

**BIOLOGICAL EFFECTS OF HUTCH COVERS IN REDUCING HEAT AND  
COLD STRESS IN INDIVIDUALLY HOUSED DAIRY CALVES**

A Thesis

by

JADE ASHLEE HABERMAN

Submitted to the Office of Graduate and Professional Studies of  
Texas A&M University  
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Chair of Committee,	Ted Friend
Committee Members,	Juan Romano
	Glenn Holub
	Mike Tomaszewski
Head of Department,	H. Russell Cross

August 2015

Major Subject: Animal Science

Copyright 2015 Jade Ashlee Haberman

## ABSTRACT

Two studies were conducted to evaluate the biological effects of hutch covers in reducing heat and cold stress in individually housed dairy calves.

The heat stress study was conducted on two farms, one in Arizona (AZ) and one in the Texas Panhandle (TX). Biological parameters were used to compare unweaned calves housed in reflectively covered hutches with calves in uncovered hutches. Average daily maximum temperature was 7.78 °C warmer ( $P < 0.01$ ) at AZ than at TX throughout the study. Internal hutch temperature of the reflective covered hutches was 2.16 °C cooler ( $P < 0.05$ ) at AZ, and 2.57 °C cooler ( $P < 0.05$ ) at TX than control hutches during the hottest 4-h portion of the day. Respiration rates at AZ were lower ( $P < 0.01$ ) for reflectively housed calves than for control calves. While housed in reflective hutches, fewer ( $P < 0.05$ ) calves were treated for ear infections than control calves and at 4 months of age, fewer calves that had been housed in reflective hutches were treated for pneumonia than control calves, possibly indicating long-term benefits. Reflective covers did not affect ( $P > 0.05$ ) weight gain or immune response to an IBR vaccination at either farm. Reflective hutch covers moderate internal hutch temperature to a degree that can affect biological function. Absence of persistent infected calves with BVD, and high antibody titers to IBR indicate the farms' vaccination and biosecurity practices against BVD and colostrum programs were successful.

The cold stress study was conducted during two consecutive winters in the Texas Panhandle. Unweaned calves housed hutches covered with 2 different materials (reflective and non-reflective) were compared during two consecutive winters (Trial 1 and Trial 2). Average daily temperature minimums were colder ( $P < 0.01$ ) during December and January of Trial 1 than of Trial 2. Internal hutch temperature was 1.22 °C warmer ( $P < 0.05$ ) in reflective hutches and 0.67 °C warmer ( $P < 0.05$ ) in non-reflective than control hutches covers during the night of both trials but, was not different ( $P > 0.05$ ) during daylight hours (0800-2000). Hutch covers demonstrated heat-retaining abilities but did not have a conclusive effect on ADG.

In conclusion, reflective hutch covers effectively moderate internal hutch temperature during sunny summer days, but not during winter.

## **DEDICATION**

This thesis is dedicated to my family. They have truly been the best support system, even from hundreds of miles away. To my parents, thank you for your continuous unyielding love, support, and sacrifice to ensure the success of your children. Mom, you are utterly amazing. You seem to have an answer for any problem I face. How you manage to take care of all of us all while working entirely too much, I will never know. Dad, your work ethic and determination are something I admire. Thank you for never letting me take life too seriously. Bailee, you know how to make the best out of every situation and I am very lucky to have you as not only my sister but my best friend. There is absolutely never a dull moment when you are around. Drew, my favorite brother, you have developed into such a caring, genuine person. I am very proud of you.

I have been so incredibly blessed to have such a wonderful family. The past few years have brought us all so close despite never getting to spend more than a couple weeks together at a time. Thank you for pushing me and never giving up on me.

## **ACKNOWLEDGEMENTS**

I would like to thank my committee chair, Dr. Friend, for all his guidance and support throughout my degree program. His mentorship and constructive criticism have influenced me more than any class I have ever been enrolled in. A special thank you to Dr. Hairgrove, as he was always willing to go above and beyond in providing unlimited advice and encouragement throughout this experience. He has been a stellar role model. Thank you to my committee: Dr. Holub always made time for my questions no matter how busy his schedule was, Dr. Romano provided valuable advice, thank you for sharing your expertise, and Dr. Tomaszewski for the support and patience.

A big appreciation to Dr. Velayudhan at the Texas A&M Veterinary Diagnostic Laboratory in Amarillo for his great patience and organization when dealing with all the samples I set into the lab. I would also like to extend my gratitude to Novartis Animal Health for the generous donation of the vaccinations used in this study.

Thank you to Dairy Fountain, especially Klaas, for being interested in our studies and so helpful to me throughout the past two years. My experience at the dairy has greatly influenced my future aspirations. To T&K Red River Dairy, especially Tim and Dr. Robson, thank you for your patience and help throughout the summer study. The attention given to improving calf welfare at your dairy was exceptional.

I want to recognize the students who helped me make the hutch covers and traveled to help me put them up (Amanda, Rovin, Itzel, Eboni, Lela, Marc). And to Lindsey, Liz, and Lauren who were not only amazing help but also extended their friendship to me and made this experience all the better. Elizabeth, thank you for taking me under your wing and always being there for me. I am truly grateful to have you as a friend. To my officemate and friend Sarah, thank you for keeping me sane through this process.

## **NOMENCLATURE**

ADG	Average Daily Gain
ANOVA	Analysis of Variance
BG	Black Globe
BVD	Bovine Viral Diarrhea
d	Day
DMI	Dry Matter Intake
h	Hour
IBR	Infectious Bovine Rhinotracheitis
kg	Kilogram
LDPE	Low Density Polyethylene
PI	Persistently Infection
PR	Pulse Rate
RR	Respiratory Rate
SE	Standard Error
THI	Thermal Heat Index

## TABLE OF CONTENTS

	Page
ABSTRACT.....	ii
DEDICATION.....	iv
ACKNOWLEDGEMENTS.....	v
NOMENCLATURE .....	vi
TABLE OF CONTENTS.....	vii
LIST OF FIGURES .....	ix
LIST OF TABLES.....	x
 CHAPTER	
I        INTRODUCTION .....	1
Implications of Heat Stress on Dairy Cattle .....	1
Implications of Cold Stress on Dairy Cattle .....	2
Housing of Dairy Calves.....	3
Justification.....	3
II       REVIEW OF THE LITERATURE .....	5
Heat Stress .....	5
Cold Stress .....	8
Immunology.....	9
Housing.....	12
III      USING REFLECTIVE HUTCH COVERS TO REDUCE HEAT STRESS ON CALVES IN POLYETHYLENE HUTCHES.....	15
Introduction .....	15
Materials and Methods .....	16
Results.....	24
Discussion .....	33
Implications .....	38

CHAPTER		Page
IV	USING REFLECTIVE HUTCH COVERS TO REDUCE COLD STRESS ON CALVES IN POLYETHYLENE HUTCHES .....	40
	Introduction .....	40
	Materials and Methods .....	41
	Results .....	45
	Discussion .....	49
	Implications .....	52
V	GENERAL CONCLUSIONS .....	53
	Environmental Stress.....	53
	Future Investigation.....	54
	LITERATURE CITED .....	56



## LIST OF FIGURES

	Page
Figure 3.1 Installed LDPE reflective hutch covers on North and South facing hutches at AZ .....	17
Figure 3.2 Illustration of method used to mount iButton temperature loggers inside the hutch to record internal hutch temperature.....	21
Figure 3.3 Antibody titers of calves housed in reflective and control calves with SE for d 21, d 42, and d 56 .....	26
Figure 3.4 Comparison of mean internal hutch temperature during a 24-h period with little cloud cover between reflective and control hutches at AZ .....	29
Figure 3.5 Comparison of mean internal hutch temperatures during the hottest 4-hour period of the day between reflective and control hutches at AZ.....	30
Figure 3.6 Comparison of internal hutch temperature between Agri-Plastic and Calf-Tel hutches during the hottest portion 4-h of the day .....	30
Figure 4.1 Installed reflective (left) and non-reflective (right) covers on South facing Model 1 hutches.....	42
Figure 4.2 Average internal hutch temperature of control, reflective, and non-reflective hutches from 2130-0530 over 7 periods with clear skies in Trial 1 .....	47
Figure 4.3 Average internal hutch temperature of control, reflective, and non-reflective hutches from 0730-0930 over 7 periods with clear skies in Trial 2.....	47
Figure 4.4 Internal hutch temperature of Model 1 and Model 2 hutches from 0700-1900 averaged over 7 days with clear skies during Trial 2 .....	48

## LIST OF TABLES

	Page
Table 3.1 Average maximum temperature and temperature difference for each month between AZ and TX .....	24
Table 3.2 Antibody titer differences at different stages of the immune response to IBR vaccination for calves housed in control and reflective hutches.....	26
Table 3.3 Antibody titer differences at different stages to IBR vaccination for male and female calves housed in control and reflective hutches at AZ .....	27
Table 3.4 Least squares mean for total weight gain (kg) during 56 day trial period for control and reflective housed calves at AZ .....	28
Table 3.5 Least squares mean for total weight gain (kg) during 91 day trial period for control and reflective housed calves at TX.....	28
Table 3.6 Least squares mean for total weight gain (kg) during 56 day trial period by sex at AZ.....	28
Table 3.7 Comparison of mean internal hutch temperature (°C) during hottest 4-h period of the day between control and reflective hutches .....	29
Table 3.8 Mean respiration rate per minute for control and reflectively housed calves with and without cloud cover at AZ .....	31
Table 3.9 Number of calves that received medical treatment and mean number of treatments administered by symptom for calves housed in control and reflective hutches at AZ .....	32
Table 4.1 Average minimum temperature for each month between Trial 1 and Trial 2 .....	45
Table 4.2 Comparison of ADG in kg for calves housed in Model 1 hutches for Trial 1 and Trial 2 .....	49
Table 4.3 Comparison of ADG between Model 1 and Model 2 hutches for control and non-reflective calves in Trial 2 .....	49

# **CHAPTER I**

## **INTRODUCTION**

The increasing demand on the agriculture industry has resulted in fewer dairies with larger herd sizes, especially in the Southwest United States. As herd size increases, farms utilize more intensive management techniques including increased record keeping of individual animals. This has allowed for genetic improvement nearly tripling average annual milk production per cow (USDA, 2007). Approximately 91.5% of large dairies (>500 cows) use conventional methods such as dry lots to manage their herds.

Confinement can reduce the animal's ability to moderate its own environment, resulting in both mental and physiological stress. Energy is thus diverted from growth and production to mechanisms that allow the animal to better cope with its environment. Therefore, providing adequate abatement from adverse conditions has become a priority for producers to ensure maximum productivity and welfare.

### **Implications of heat stress on dairy cattle**

Heat stress costs the United States dairy industry \$879 million to \$1.5 billion annually (St-Pierre et al., 2003). As commercial dairies continue to expand across the Southwest United States, efficient methods to combat heat stress become essential for success in the hot humid or arid environment. The Southwest United States, including Arizona and the panhandle of Texas, are classified as sub-humid and semiarid and cattle face high ambient temperature and humidity during the months of summer. Dairy cows are selectively bred for higher milk production and become more susceptible to heat stress due to higher metabolic activity. The trend in the United States dairy industry has increased herd size along with milk production per cow. The average dairy cow on a large commercial farm (>500) produces 10,290 kg of milk annually (USDA, 2007).

Dairy cattle are best suited for environments from 16-25 °C. When temperature exceeds this range, milk production, immune function, and reproductive efficiency

decrease and disease incidence increases in lactating animals (Hahn, 1999; Collier et al., 2006; Tao et al., 2012). Heat stress in Texas reduces milk yield by 2,007 kg per cow annually (St-Pierre et al., 2003). Heat stress also reduces the conditions in utero and calves born to heat stress dams have decreased birth weight, weaning weight, and efficiency of IgG absorption for passive immunity in comparison to calves not born to heat stress dams (Reynolds et al., 1985; Dreiling et al., 1991; Wu et al., 2006; Tao et al., 2012). Heat stress slows weight gain and delays the heifer's sexual maturity, increasing her maintenance costs and delaying generation of revenue from milk production (Bungert, 1998).

### **Implications of cold stress on dairy cattle**

Due to their higher metabolic rate, dairy cows are more susceptible to heat stress than cold stress. But, once outside of the cow's thermal neutral zone, low ambient temperature negatively affects reproduction and milk production (Brouček et al., 1991). Cold animals must spend more energy maintaining body temperature, diverting it from growth and production. Cold temperature increases dry matter intake (DMI), which increases the rate of feed passage through the gastrointestinal tract. As the rate of passage increases, it reduces the efficiency of digestion (NRC, 2001). As a result, cold stress increases feed costs while decreasing profitability of lactating animals.

In the dairy calf, winter increases morbidity and mortality (Godden et al., 2004). Winter losses are greatly influenced by wet and windy weather (Nonnecke et al., 2009). Morbidity rate of calves born in winter in an arid environment were 4% higher than in other seasons (Mellado et al., 2013). The effect of cold ambient temperature on the immune system has been illustrated in other species (Nonnecke et al., 2009). Frank et al. (2003), found that pigs briefly (5 d) exposed to cold had an increase in stress-related hormones acetylcholine and cortisol, and increased levels of proinflammatory cytokines. As maintenance energy is increased for heat generation, energy is diverted away from other body functions including growth and immunity (Drackley, 2005). Given adequate

nutrition, a calf can adapt to fluctuations in its environment but without nutritional supplement calves, particularly in sustained cold environments may be more affected.

### **Housing of dairy calves**

More than half the calves in the United States are housed in individual polyethylene hutches. Individual housing can limit disease transmission and allows for biocontainment. Calves reared in hutches have been found to have lower death losses than other housing methods (Lance et al., 1992). Outdoor individual hutches require less labor than individual indoor housing and alleviates inadequate ventilation by allowing more airflow (Jorgenson et al., 1970). Airflow is not only important in reducing internal hutch temperature but also with the buildup of ammonia. Polyethylene hutches are made for year-round use and their design causes the hutch to retain solar radiation (Coleman et al., 1996). During winter the heat retention is beneficial but, during summer shade provided by the hutch is less beneficial due to its absorption of energy and the resulting higher internal hutch temperature. Shade cloth and other structures have been used to shade hutches and lower internal hutch temperature but the possibility of facilitating the growth of harmful bacteria has raised concern. Shade cloth prevents the UV rays from reaching the bacteria, preventing its antimicrobial properties (Coleman et al., 1996; Reed et al., 2004). In response, an individual reflective hutch cover, that is affordable and durable, has been developed. It has been found to significantly lower internal hutch temperature, but the biological significance of its effect on the calves has yet to be determined (Friend et al., 2014).

### **Justification**

As the U.S. dairy industry moves towards fewer farms with larger herds, more intensive management techniques are utilized. Confined animals are less able to moderate their microclimate independently and rely heavily on the producer to ensure suitable environmental conditions. The increased metabolic rate of high milk producing cows renders them most susceptible to heat stress. Many cooling methods currently

used in dairy management focus on the lactating animal, neglecting the dry cows and calves. Studies are needed to develop efficient methods to combat heat stress in calves as well as adults. Improvements will increase the profitability of dairy operations while increasing the comfort of the animals.

Thermoregulation in calves is less understood than in the adult animal. Due to a smaller relative body surface area to mass and lower production of metabolic heat, calves are more susceptible to cold stress than heat stress, but either is detrimental without abatement techniques. Heat and cold stress creates residual effects on the calf. Heat stress reduces the utero environment for the calf, decreasing immune function, birth weight, ability to absorb colostrum, and possibly reducing yield at its first lactation (Reynolds et al., 1985; Dreiling et al., 1991; Wu et al., 2006; Tao et al., 2012; Monteiro, 2013). By decreasing weight gain, heat and cold stress can delay sexual maturity. In turn, calving at 26 months instead of 24 months, each heifer has a \$90 higher feed cost and at a 30% cull rate, it requires 12 more replacement heifers per 100 head of cows annually. Milk production is in turn delayed for the heifer, increasing the amount of time before the producers will receive a return from the animal. The longer interval also increases the amount of replacement heifers needed per hundred head of cows with a cull rate of 30% each year (Bungert, 1998).

While there are multiple methods to house dairy calves, individual hutches or pens are the most popular in the United States (USDA, 2007). Development of a durable reflective hutch cover used in conjunction with individually housed calves may be most effective in reducing heat stress. Friend et al., (2014), found that an aluminized 3.0 mil polyethylene cover can significantly reduce internal hutch temperature during hot periods but its efficacy on calf health has yet to be determined. Investigating the biological significance of the reflective hutch covers is necessary to determine if its use serves as a viable option for cold and heat abatement in dairy calves. Covers may positively influence weight gain, respiration rate, and possibly immune function in dairy calves, improving health and reducing input costs for replacement heifers.

## **CHAPTER II**

### **REVIEW OF THE LITERATURE**

Understanding the effects of thermal stressors on the growth and health of neonatal replacement heifers holds great potential for improving the dairy industry. The implications caused by prolonged thermal stress are not be immediately apparent. Few heat and cold abatement techniques are used for calves housed in individual polyethylene hutches. Limiting heat and cold stress using economically feasible methods can support the dairy industry by best meeting the needs of the growing animal.

#### **Heat stress**

Within the thermoneutral zone (TNZ), heat is dissipated at equilibrium with internal heat production and heat gained from the environment (Figure 2.1). A calf's TNZ ranges from 15 and 25°C but can be affected by age, hair coat, and feed intake (NRC, 2001). Both cutaneous and internal temperature sensors work together to maintain constant body temperature. Processing this information occurs in the hypothalamus which makes appropriate changes using the cardiovascular, respiratory, digestive, and endocrine systems to encourage heat loss. The main regulator of body temperature within the TNZ is the vasoconstriction and dilation of peripheral blood vessels (Charkoudian, 2010). As temperature begins to exceed the TNZ, heat cannot be dissipated from the body as quickly as it is metabolically produced and/or acquired from the environment. Body temperature in turn increases, and the animal must then divert energy from production and growth towards temperature regulation. Maintenance requirements increase 20% during thermal stress (Bungert, 1998).

The calf begins to respond physiologically to maintain thermostasis as temperature exceeds 26.7 °C at 60% relative humidity (RH). Mechanisms of negative feedback triggers vasoconstriction of peripheral blood vessels which reduces uptake of additional heat as the environmental temperature exceeds body temperature (Hales et al., 1985). At this point, evaporation serves as the main source of heat dissipation because

less energy is required for the evaporation of moisture and the rate of sweating increases, then plateaus, with temperature and radiant energy (Finch, 1985). Evaporation occurs as sweat on the skin's surface and in the lungs and nasal passages. In adult cattle, standing behavior increases with thermal temperature (Berman, 2005). By standing, more of the body surface is exposed to the air, possibly increasing evaporative cooling. In calves, more time is spent lying during high ambient heat, possibly because calves have less stamina than adults, and muscle energy stores are depleted faster. Sweating rates in *Bos Taurus* cattle significantly increases from 15 to 20 °C, reaching its maximum before 30 °C (Silvanikove, 2000). Respiration rate also increases as the thermal environment exceeds body temperature. As water changes phases from liquid to gas, energy is lost, (0.580 Kcal/g at 35 °C) which cools the skin (Schmidt-Nielson, 1997). Water intake is increased to sustain hydration for evaporative cooling and decreasing body temperature directly (Stermer et al., 1986). Systemic indicators of heat stress including heart rate and cortisol are increased in the calf as it attempts to adjust to an increased heat load (Neuwirth et al., 1979). Respiration rates and rectal temperature can be used for rapid detection of heat stress in calves. Basal respiration rates are approximately 20 breaths per minute in cattle and increase to 40 breaths per minute as the animal begins to pant to increase evaporative cooling. Severe heat stress can be characterized by respiration rates above 150 breaths per minute (Silvanikove, 2000). Normal body temperature for dairy calves is approximately  $38.6 \pm 1$  °C (McDowell, 1972). Hyperthermia is indicative of the animal's inability to dissipate the necessary amount of heat to retain homeostasis in hot environments.

Environmental conditions such as humidity, wind, and cloud cover also determine the effectiveness of heat dissipation. Humidity increases as more water vapor saturates the air. As humidity increases, it becomes more difficult for water to vaporize due to the increase downward pressure. For this reason, it becomes extremely difficult for the animal to cool itself as temperature and humidity rise. Wind can increase the effectiveness of evaporative cooling by maintaining a supply of unsaturated air around the animal. The amount of radiant energy from the sun can also be affected by cloud



cover (Graham, 1999). Radiant energy excites the atomic particles in the mediums that absorb it, which change it to thermal energy, and increases its temperature. Objects with higher temperatures also radiate more energy (Schmidt-Nielson, 1997). Dark surfaces radiate and absorb more heat than light ones at the same temperature (Silvanikove, 2000).

Heat stress on the dam directly affects the calf. Heat stress on the lactating dairy cow is well documented and dramatically decreases milk production, reproductive performance, and increased instances of disease (Collier et al., 2006). During the dry period before parturition, heat stress decreases subsequent milk production and compromises fetal growth and development (Tao et al., 2011). Calves born to heat stressed dams had decreased birth weight, weaning weight, and efficiency of IgG absorption for passive immunity in comparison to calves not born to heat stress dams. Decreased birth weight can be attributed to a number of factors including decreased gestation length, decreased dry matter intake (DMI), and reduction of oxygen and nutrient exchange between dam and calf (Reynolds et al., 1985; Dreiling et al., 1991; Wu et al., 2006; Tao et al., 2012). Use of shades, ventilation, sprinklers and fans to cool lactating cows have been found to offset the effects of heat stress (Armstrong, 1994).

Few heat abatement techniques are used for young stock housed in individual polyethylene hutches. Insufficient water supply limits feed intake of the calves by preventing proper nutrient digestion and absorption and thermoregulation preventing calves from reaching target weight gain (Winchester and Morris, 1956). Inadequate water supply calves will also result in dehydration and even death. Heat stress can have both short-term and long-term effects. If neglected during heat stress, calves can experience reduced growth rates which increases age of puberty and delays breeding and calving (Bungert, 1998). It can also decrease milk yield at the heifer's first lactation (Monteiro, 2013). Without heat abatement, females are kept open an average of 25.6 days longer than during the rest of the year in Arizona and 53.9 days longer in Texas (St-Pierre et al., 2003). As age of calving increases, more money is spent feeding the heifer, more replacements are needed to maintain the same cull rate, and milk is not being

produced. Calves can optimally adjust to increased thermal conditions with increased plane of nutrition and sufficient water supply (Bungert, 1998).

### **Cold stress**

Calves are more susceptible to cold stress than heat stress (Bianca, 1976; Mellado et al., 2014). There are a variety of factors that contribute to the increased susceptibility to cold stress which include thin skin, small amounts of subcutaneous fat and the calf producing significantly less metabolic heat than an adult. They also have a greater relative surface area to body mass (Olson et al., 1980; West, 2003). The greater the surface area to mass ratio, the more heat can be lost to the environment. During low ambient temperatures, at birth a calf loses great amounts of body heat due to the evaporation of amniotic fluids and exposure. Calves with lower birth weights are more susceptible to hyperthermia than heavier calves (Azzam et al., 1993). Heat loss can be evaporative from respiration or wet skin or can occur in non-evaporative ways such as radiation, conduction, and convection. Rate of heat loss can be influenced by hair coat, wind, and precipitation.

Below the lower critical temperature of the TNZ, the calf must generate more heat to maintain its body temperature. Heat loss can be reduced by a variety of mechanisms including piloerection of its hair coat and by vasoconstriction of its peripheral blood vessels. Muscle can produce heat in the body by shivering and by increasing physical activity. Calves can acclimate to cold temperatures by increasing respiration rate. In an effort to maintain body temperature, blood is pumped faster, increasing the rate the lungs must pump oxygen to the blood (Mellado et al., 2014). Heat generation mechanisms are energy costly. Calves housed in -4 °C have higher maintenance energy requirements than calves housed at 10 °C; therefore, without nutritional supplementation weight gain is decreased as is body temperature (Scibilia et al., 1987).

Mortality rates peak during the winter season (Godden et al., 2004; Mellado et al., 2014). Similar to high ambient temperature, low ambient temperature also impairs

absorption of immunoglobulins from colostrum and can lower the quality of colostrum (Olsen et al., 1980; Shearer et al., 1991; Beam et al., 2009).

## **Immunology**

Heat stress can cause deviations from the normal immune response. Under optimal conditions, the body has a variety of defenses against pathogens including physical barriers, innate, and acquired mechanisms. Physical barriers are the first line of defense and include the skin, but also include actions of self-cleaning such as coughing and sneezing. Innate immunity supports the physical barriers and consists of rapid responders that work to actively destroy invading microorganisms as well as to mark them for destruction by the acquired immune system. Antigen presenting cells, such as macrophages and dendritic cells, have antigen specific receptors. These cells present the antigen to lymphocytes (B and T cells), which also have antigen specific receptors. The activation of specific lymphocytes is marked by cell proliferation of cells that either function in antibody production or cell-mediated immunity (effector cells). Both pathways also produce memory cells. Antibody producing cells and effector cells aid in the current invasion, while memory cells remain in circulation to mount a more efficient response in the event the same invader is encountered again. Vaccinations also produce memory cells to protect the organism against future infection. Antibodies are also acquired by passive immunity (Tizard, 2009).

Calves receive vital protection against disease through passive immunity from colostrum at birth. Colostrum contains more protein, immunoglobulins, fat, vitamins, and minerals than milk. The calf's ability to absorb immunoglobulins in the colostrum decreases drastically within the first 36 hours of life. Intestinal permeability to colostrum is highest at birth and drastically declines within the first 6 hours of birth (Tizard, 2009). Failure of passive transfer (FPT) is defined as the calf having an IgG serum concentration level of less than 10 mg/mL (Stilwell and Carvalho, 2011). At 24 to 48 hours of age, mortality rates are doubled for calves with IgG levels under this threshold (Tizard, 2009). FPT is associated with increased disease susceptibility,

hindered reproductive ability, and increased mortality (McGuirk and Collins, 2004). It is suggested that dairy calves be removed from the dam within 2 hours of birth and fed a volume of 10% of the calf's body weight of clean colostrum using a nipple bottle or esophageal tube feeder (McGuirk and Collins, 2004). Studies have indicated that there is no difference in passive immunity when large volumes (3 L) of colostrum are fed using either method (Godden et al., 2009). Colostrum should contain immunoglobulin levels of at least 50 g IgG/L (McGuirk and Collins, 2004).

Bovine colostrum is rich in lymphocytes, half of which are T cells. Lymphocytes function in both humoral and cell mediated immunity. Lymphocytes can survive up to 36 hours in the intestine of the calf and are rapidly absorbed into the bloodstream. Ingestion of colostrum accelerates the activation of calf lymphocytes and increases the antigen processing and presenting abilities by its monocytes. The ingestion of colostrum also inhibits the calf from mounting its own B cell immune response, although T cell responses are largely unaffected. One proposed mechanism of how maternal antibodies inhibit B cells from mounting an immune response is by masking the antigen's epitope. This prevents recognition of the antigen by the specific B cell. For this reason, maternal antibody titers must fall below a critical threshold before a particular vaccine can be effective. Although it is likely that there will not be an initial immune response to a vaccination while maternal antibody levels are still high; the anamnestic response to a booster vaccination can be higher than in animals only vaccinated after maternal antibodies have declined. Exposure to an antigen while maternal antibodies are present may prime future responses when encountered again (Menanteau-Horta et al., 1985). Other immune functions do not appear to be directly influenced by maternal antibodies such as T cells and IgA.

IgA antibodies serve as part of the first line of defense for the mucosal surfaces. IgA antibodies function mostly independent from other humoral immune responses to prevent over stimulation of the immune system. This is done in a variety of ways including the neutralizing the antigen, inducing the release of anti-inflammatory cytokines, and limiting the activation of dendritic cells (Corth sy, 2007). Due to its

independent nature, specific maternal antibody titers do not influence IgA titers. Measuring IgA titers can serve as a suitable method to examine immune response without maternal antibody interaction.

Immune function can be used to determine how effective a vaccine is under particular conditions. By quantifying antibody response, quality of immune function can be determined. But because maternal antibodies inhibit the calf from synthesizing its own antibodies, traditional vaccination methods such as subcutaneous and intramuscular routes are often unsuccessful.

Intranasal (IN) vaccines have the ability to prime the mucosal immune system (IgA) with little interference from secretory and maternal antibodies (Chase et al., 2008). IN vaccinations often produce low or no antibody titers detectable by serology but have been found to provide immunity that lasts for months (Ellis et al., 2007). IN vaccinations are commonly used for Infectious Bovine Rhinotracheitis (IBR), Bovine Respiratory Syncytial Virus (BRSV), and Parainfluenza3 (PI3).

Dairy calf managers employ a variety of methods to limit disease transmission in neonates including individual housing and IN vaccinations at birth. Dairy heifers have a mortality rate of approximately 3.8% (Perez et al., 1990). Some of the most common afflictions the dairy calf will experience are scours and respiratory illness such as pneumonia. Scours is most prevalent within the first 30 days of a calf's life and can be caused by pathogens in the environment and in unpasteurized milk. Calves are also more susceptible to scours after the first occurrence (Perez et al., 1990). Providing adequate water supply and maintaining caloric intake is extremely important during this time to limit dehydration (McGuirk, 2008).

Respiratory illness affects approximately 15% of dairy calves before weaning and its prevalence increases over scours after 30 days of age (McGuirk, 2008; Stanton, 2009). Early detection of pneumonia is essential to limit the subclinical effects on the calf including weight gain.

The trend in growth of the U.S. dairy industry has resulted in fewer farms with larger herd size. Large herd size (>500) and the purchasing of animals have been found

to be risk factors for herds with Bovine Viral Diarrhea (BVD) (USDA, 2007). Although the trend for testing replacement heifers increases as herd size increases, only 21.2 % of dairies with herd size >500 routinely test heifer replacements to determine if the animals are persistently infected (PI) to BVD (USDA, 2007). BVD results in economic losses due to decreased milk production and reproductive performance in dairy cows.

Persistently infection occurs in utero when the fetus is exposure to BVD before 125 days of gestation (Hanon et al., 2014). During the first 125 days of gestation, the calf has yet to become immunocompetent. PI cattle continuously shed the BVD virus and will continue to do so their entire life. They also produce PI offspring. PI calves have a death rate of 50% during the first year of life (Smith, 2009). These calves are also predisposed to infection which alters the ability of the calf's immune system to respond to vaccinations.

PI status can be detected using virus isolation and antigen capture ELISA. There is no specific treatment for BVD and is best prevented with BVD vaccination programs and strict biosecurity. BVD vaccination is of particular importance during early gestation where the fetus is most susceptible to the virus.

## **Housing**

Individual hutches are a common way to house preweaned dairy calves in the United States. A total of 67.8% of U.S. dairies utilize individual hutches or pens (USDA, 2007). Individual, outdoor hutches provide many benefits to the calves and the producers. They require less labor than individual indoor housing and alleviate inadequate ventilation by allowing more airflow (Jorgenson et al., 1970). Hutches also provides biosecurity and biocontainment. Preweaned calves reared in hutches have been found to have lower death losses than other housing methods (Lance et al., 1992).

Polyethylene hutches are used year-round and their design causes the hutch to retain solar radiation (Coleman et al., 1996). While the heat retention is beneficial during the winter, the shade produced by the hutch becomes less beneficial to the calf as the temperature humidity index (THI) increases. The hutch emits more radiant heat to

the calf and during high THI, evaporative cooling, such as sweating and panting, by the calf is not as effective in lowering body temperature due to the high moisture content of the air. Hutch set-up and orientation can be effectively managed to optimize conditions inside the hutch year round. During summer months, airflow can be enhanced by opening hutch vents, preventing build-up of bedding, and placing a block underneath the back wall (Moore et al., 2012). Elevation of the hutch reduces temperature, respiration rates, carbon dioxide levels, and airborne bacteria levels (Hill et al., 2011). Maintenance of clean, dry of organic bedding reduces evaporative and conductive heat loss during cold weather (Eastridge, 2014).

Shade structures have been used to supplement individual polyethylene hutches during summer months. These structures improve calf comfort by decreasing ambient temperature, body temperature, and respiration rate (Spain and Spiers, 1996). While blocking the sun reduces solar radiation, the potential for increased bacterial counts due to blocking the sun's ultraviolet (UV) rays, has raised concern (Coleman et al., 1996; Reed et al., 2004). Bedding collected from shaded calves had higher counts of both total and fecal coliform. While Coleman et al. (1996) reported that this did not appear to adversely affect calf health; periods of increased rainfall may cause increased instances of disease.

In an effort to sustain the benefits of the sun's UV rays, Carter et al. (2012) found that an individual insulated reflective hutch cover was successful in significantly reducing internal hutch temperature. Although the reduction in temperature did not result in an increase in average daily gain; respiration rate was decreased which indicates an improvement in calf comfort. Unfortunately, the reflective material used was bulky, expensive, and fragile. Both Binion et al. (2014) and Friend et al. (2014) found that various aluminized polyester films were successful in reducing internal hutch temperature in comparison to uncovered hutches. Friend et al., (2014) have developed a more economical version of the reflective polyethylene hutch cover with increased longevity. This reflective cover significantly reduced internal hutch temperature but the

biological significance of the cover on the dairy calves has yet to be determined (Friend et al., 2014).



# **CHAPTER III**

## **USING REFLECTIVE HUTCH COVERS TO REDUCE HEAT STRESS ON CALVES IN POLYETHYLENE HUTCHES**

### **Introduction**

The objective of this study was to determine the effects of reflective hutch covers on dairy calf body weight, respiration rate, health (disease incidence), and possible immune function. This study also considered the colostrum program of the farms and the presence of PI's in the herd. The effects of heat stress are often associated with the lactating animal where milk production is reduced, dry matter intake decreases, and fetal growth and development in pregnant animals is compromised (Collier et al., 2006; Tao et al., 2012). The young stock often receives minimal heat abatement attention; and therefore, the literature on heat stress in the neonate is less comprehensive than for the adult. There is a possibility that addressing heat stress in the neonate will identify economic and welfare related benefits.

Previous research by this lab identified the significant heat reducing abilities of reflective hutch covers on internal hutch temperature. By blocking and reflecting radiant energy, internal hutch temperature is kept cooler than in uncovered hutches. The biological significance of this heat reduction was briefly examined by Carter et al. (2012) where he found that although weight gain was not affected by the reflective covers, respiration rate was in fact lowered in the covered animals. Unfortunately the material used in that experiment was bulky, expensive and lacked structural integrity. The use of reflective low-density polyethylene (LDPE) material resolves the issues associated with the old material. The current cover has been designed to be light-weight, easy to install, and inexpensive enough to be disposable. Its practicality for heat abatement in a commercial dairy setting was evaluated in this study by measuring biological functions during a period of high ambient temperature.

## **Materials and methods**

Two large commercial dairy farms were used in the study; one near Plainview, Texas and one near Stanfield, Arizona. Animal care and use was approved by the Institutional Animal Care and Use Committee of Texas A&M University (AUP # 2014-125). The study began in June and ended the second week of August 2014. The Texas dairy had 56 heifer calves and the Arizona dairy had a combination of 66 bull and 51 heifer calves that were born in close enough intervals to be enrolled in the study. Calves on both farms underwent the same general protocol for the study. Upon birth, each calf was removed from its dam before consuming colostrum. Each calf received two feedings of pooled colostrum via stomach tube before moving to the hutch, one within the first hour from birth and the second within four hours after birth. Calves were moved to their hutch at 8-h of age at AZ and 48-h of age at TX. Calves were housed in individual polyethylene hutches Calf-Tel (Calf-Tel Pro, Hampel Corp., Germantown, WI, USA) at the Texas farm (TX) and Agri-Plastics with the top ventilation slide removed (EXL, Agri-Plastics, Grassie, Ontario, CA) at the Arizona farm (AZ). Both farms utilized a 1.2 x 1.8m outdoor wire pen attached to the hutch that was modified from cattle panel. Calves remained under the respective farms' protocol for feed, water, and medical care. Hutches at AZ were placed in a row and the opening of the hutch alternated from south to north. Hutches at TX were placed in a row, all facing south. The covers were installed before the calves entered the hutches and alternated between 5 reflective and 5 control hutches down the row at both locations.

The reflective cover was installed on the outside of the hutch covering the top, sides and back of the hutch. The cover was constructed from 3.0 mil (LDPE) with an aluminized layer facing the outside. It was approximately 1.8 x 3 m and had a 0.13 m sleeve along its 1.8 m sides. It was secured with a 1.85 m long Schedule 20 polyvinyl chloride (PVC) pipe that was inserted into its side sleeves. Each piece of PVC pipe was attached to 2 custom bungee cords with hooks that attached underneath the hutch to anchor it. The installed reflective hutch cover is depicted in Figure 3.1. The reflective

polyethylene covers were adjusted occasionally through the study for proper fit on the hutch.



Figure 3.1. Installed LDPE reflective hutch cover on North and South facing hutches at AZ

Calves were assigned to treatment or control groups as they were moved into the hutches. Each farm had a calf housed in an uncovered hutch (control) for every calf housed in a reflective covered hutch. At AZ calves were moved in groups from the nursery to the hutches at two times throughout the day. Calves of the same sex were placed in adjacent hutches so that groups of males alternated with females. Criteria for calf enrollment in the study included calves born without dystocia and physical

deformity. The enrollment period at AZ was 3 days and 5 days at TX, resulting in a 3 and 5-day age range among calves respectively.

#### *Persistent infection of BVD*

In order to rule out PI of BVD as a confounder an ear notch was taken from each calf at birth. PI individuals are exposed to the antigen (BVD in this experiment) in early gestation. This test indicated herd disease status for BVD. The right ear was cleaned with alcohol before the sample was taken. A small commercial ear notcher was used to take the sample and the sample was stored in a microcentrifuge tube. The ear notcher was rinsed with water and disinfected with Chlorhexidine diacetate (Nolvasan) between calves. The samples were kept refrigerated before being sent to the Texas A&M University Veterinary Diagnostic Laboratory in Amarillo, Texas in a cooled shipping container.

Antigen Capture ELISA was used to determine presence of BVD in the sample. A commercially available BVD antigen capture ELISA kit was used to determine presence of BVD antibodies (BVDV PI X2®, IDEXX, Hoofddorp, The Netherlands). The procedure followed the manufacturer's instructions. Ear notches were prepared with a soak buffer and were tested for BVD antigen using E<sup>ns</sup>-capture ELISA. Detection antibodies were applied to wells of the microtiter plate coated with E<sup>ns</sup> MAbs. Positive and negative controls were added to duplicate wells. The plate was incubated for 1 hour at 37°C then washed. Conjugate and substrate were then added. The optical density values (ODs) were measured at 450 nm, and corrected by subtracting the mean negative control OD from the sample and positive control ( $COD = OD^{obtained} - \text{mean } OD^{(negative \text{ or } positive \text{ controls})}$ ). A  $COD > 0.30$  was classified as positive. Calves that tested positive were removed from the study.

#### *Antibody response to IBR vaccination*

At birth (day 0), before receiving colostrum, two blood samples were collected via jugular venipuncture from each calf. One was a 7 ml draw Vacutainer® tube with no

anticoagulant (Beckton Dickinson, Lincoln Park, NJ). The second was a 2 ml draw Vacutainer® tube containing 3.6 mg ethylenediaminetetraacetic acid (EDTA) (Beckton Dickinson, Lincoln Park, NJ). The EDTA containing tube was inverted several times after blood collection to prevent coagulation. This sample determined if a calf was exposed to IBR during late gestation. Three weeks after the birth of the calves enrolled in the study (day 21), blood was again collected using the same procedure used at birth. After sampling each calf received commercially available killed BHV-1 (Vira shield 4, Novartis AG, Basel, Switzerland) in a 5 ml subcutaneous dose in the neck. This sample quantified the presence of IBR antibodies after the calf had received colostrum. Three weeks post vaccination (day 42), a third set of blood samples was collected using the same procedure used at birth, followed by a booster vaccination of Vira shield 4 (Vira shield 4, Novartis AG, Basel, Switzerland), in a 5 ml subcutaneous dose on the neck. This sample served as the initial immune response. Two weeks post booster vaccination (day 56), a fourth set of blood sample was taken. This sample determined the anamnestic immune response to the booster vaccination.

Colostrum supplied the calves with passive immunity to IBR from maternal antibodies. Maternal antibodies have the potential to neutralize the IBR antigen from the vaccination and can prevent the calves from producing its own antibodies to IBR. Measuring the calves' immune response to a foreign antigen would have avoided the potential interference with maternal antibodies. But because the study was conducted at commercial dairies and included replacement heifers, the potential risk of using a foreign antigen was unknown. An antigen was chosen that was part of the farms' vaccination programs after approval by their veterinarian.

The EDTA containing sample tubes were kept in refrigeration through transit and frozen for storage at Texas A&M University in the case extra samples were needed. Blood samples in the tubes containing no anticoagulant were refrigerated and shipped to the Texas A&M University Veterinary Diagnostic Laboratory in Amarillo, Texas. Upon arrival to the Texas A&M University Veterinary Diagnostic Laboratory, blood samples were analyzed via virus neutralization for Bovine Herpesvirus (BHV-1) in serum.

BHV-1 causes IBR and is used for clinical diagnosis. A constant predetermined titer of BHV-1 was added to serial dilutions of serum in a 96-well microtiter plate and incubated at 37 °C for 1 hour. After incubation Madin-Darby bovine kidney (MDBK) cells (ATCC CCL-22) were applied to the plate and incubated further for 2-3 days. Following incubation the MDBK cells were microscopically examined for cytopathic effects (CPE). The reciprocal of the serum sample with the highest dilution with no CPE was determined as the neutralizing antibody titer. Test validation for the assays included back titration, positive, and negative controls. A negative result was considered a titer <4 and above was considered positive for BHV-1 antibodies. Positive titer values were transformed by taking the log base 2 of the titer value to normalize the data. Calves that tested positive for IBR in the pre-colostral sample were removed from the study. This indicated the calf had been exposed to maternal IBR antibodies while in utero. Collection of all three blood samples was also a prerequisite for a subject to be included in the study.

#### *Weight gain*

Weight was measured for each calf at birth and then at day 56 at AZ and 91 at TX. Weights were analyzed separately at each farm to determine weight gain of each calf during the period it was housed in the hutches. Weight gain of control calves were compared to reflectively housed calves at both farms. Weight gain of male and female calves was also compared at AZ.

#### *Internal hutch temperature*

The iButtons (iButton, model 1921G, Maxim Integrated Products, Sunnyvale, CA, USA) was used to collect temperature at 30 minute intervals in a sample of both the control and treatment hutches. Calibrated iButtons in duplicate were set at calf level when lying (0.3 m above the ground). The iButtons were placed in a 1.8 mm wide groove cut into a piece of foam insulation. The foam served to insulate the iButton from the wall of the hutch. Wire mesh was used to cover the insulated iButtons to prevent the calf

from tampering with it. The insulated and protected iButtons were fastened to the inside of the hutch (Figure 3.2). Radiant heat was recorded by placing a calibrated into a flat-black-painted-table tennis ball, which was then mounted on the end of a wooden dowel. Black electrical tape was used to seal the iButton in the ball. Two painted table tennis balls were placed in full sun at each location. Ambient temperature was recorded by placing a calibrated iButton under a completely shaded feed bunk. The iButtons under the feed bunk were mounted in the foam block as described above. The iButtons were secured to the feed bunk using duct tape. The iButtons allowed temperature differences to be determined between treatment and control hutches at both farms. Treatment differences were also determined between Calf-Tel Pro and Agri-Plastic EXL hutches at AZ.



Figure 3.2. Illustration of method used to mount iButton temperature loggers inside the hutch to record internal hutch temperature.

### *Respiration rates*

Respiration rates were collected on day 21, 42, and 56 of the study. This was done during the hottest portion of the day by observing the calf, while the observer stood several meters away, from the hutch. Breaths were counted for 30 seconds for each calf in the study. Respiration rates were collected on three different days in the early afternoon AZ. Due to cool temperatures in TX on the days when respiration could be measured, respiration rates were not collected.

### *Medical treatment*

In order to help determine if the reflective covers have the potential to improve calf health, medical records were obtained for each calf after the end of the study. Treatment records for pneumonia from birth to 11 months of age were obtained only for calves at AZ. Treatment for medical issues requiring antibiotics, such as ear infections and pneumonia, were recorded by the farm's staff. Records were compared between treatment and control calves at AZ to determine whether calves housed with reflective covers have decreased incidences of medical treatment. TX medical records were not utilized because of the cool conditions and difficulty obtaining reliable medical data.

### *Weather data*

Weather information was obtained from the Weather Underground website ([www.wunderground.com](http://www.wunderground.com)) which sourced the weather for AZ from the Casa Grande Municipal Airport and for TX from the Hale County Airport.

### *Statistical analysis*

Data from each farm were analyzed independently because of the major climatic differences using JMP® (JMP® Statistical Software Package, ver. 11.0, SAS Institute, Cary, NC, USA). Transformed titers (logbase 2) for pre-colostral, baseline, initial immune response, and anamnestic immune response were separately analyzed using Kruskal-Wallis, a non-parametric analysis of variance. Kruskal-Wallis was used



because the data had a non-normal distribution found by using the Shapiro-Wilks Test. The difference between antibody titers for calves housed in control (n = 46) and reflective (n = 36) hutches was determined between the starting IBR titers received from colostrum (d 21) and initial immune response (d 42), the starting IBR titers received from colostrum (d 21) and anamnestic immune response (d 56), and initial immune response to anamnestic immune response.

Antibody titers for the calf's IBR antibody levels at d 21, the initial, and the anamnestic immune responses were also compared between male and female at AZ.

Difference in weight gain between control and reflective covered calves was determined using a 2 sample t-test. Weight gain was analyzed separately between farms. Weight gain was also analyzed separately between males and females at AZ. Weights are reported as the least squares mean with the standard error of the mean.

Five control and 5 reflective hutches were fitted with calibrated iButtons in duplicate at AZ, and 4 of each at TX. The temperature recordings from these hutches were divided into three periods: d 0 to d 21, d 21 to d 42, and d 42 to d 56. During each period the iButtons from the control hutches were averaged 30 minute reading. The same procedure was used for reflective, ambient, and black globe iButtons. Selected periods (max daily temperature, nightly temperature) for each period's temperature data set for control, reflective, black globe and ambient iButtons were analyzed using ANOVA followed by LSD.

Medical treatments were summarized by number of calves treated per illness type and mean number of treatments per calf, with standard deviations. Proportions of calves diagnosed with pneumonia at AZ were compared between reflective and control calves using Chi-Squared test. Fisher's Exact Test was used for the proportion of calf deaths between reflective and control calves due to the number of deaths in the reflective group of calves being less than 5. Reliable medical treatment data from TX was not available.

All data were assessed for normality of distribution using the Shapiro-Wilks Test and included the removal of outliers. Data were also assessed for equal variances using the Brown-Forsythe Test. Standard errors are presented with each mean unless

otherwise indicated.

## Results

Thermal conditions varied greatly between locations. The average daily temperature maximum at AZ was warmer than TX by an average of 7.78 °C throughout the study (Table 3.1). The extremely low temperatures in TX were unexpected. Temperature ranged from 30.56-43.89 °C at AZ and 22.22-38.33 °C at TX. TX also had a greater temperature variance for the study period. Due to the significant differences ( $P < 0.001$ ) in temperature data from each location, the farms were analyzed separately. AZ also entered the monsoon season during the latter part of the study. The monsoon season caused an increase in cloud cover and precipitation which decreased daily temperatures and radiant heat during the latter part of the study.

Table 3.1. Average maximum temperature and temperature difference for each month between AZ and TX. Average monthly max temperatures were significantly different between AZ and TX.

Average month temperature °C	AZ °C	TX °C	Temperature Difference °C	p-Value
June (d 0-d 21)	39.89 ± 0.55	30.76 ± 0.55	9.13	< 0.001
July (d 21-d 42)	41.06 ± 1.32	30.23 ± 1.32	10.82	< 0.001
August (d 42-d 56)	36.77 ± 0.41	33.39 ± 0.42	3.38	< 0.001
			Average maximum monthly temperature difference °C	7.78

### *Persistent infection of BVD in herd*

All calves sampled in the study tested negative via antigen capture ELISA for BVD. AZ had a total of 91 calf ear notches tested for BVD and TX had 32.

### *Antibody titer to IBR vaccination*

Each set of blood serum samples (d 0, d 21, d 42, and d 56) were analyzed via virus neutralization for IBR titers. Blood serum samples at AZ from d 0 indicated 3.3 % (n = 91) of calves tested positive for IBR antibodies. At TX 3.2% of (n = 31) blood serum samples tested positive. Blood serum samples at d 21 served as the immunological baseline for IBR antibodies. All calves tested positive for IBR antibodies in the d 21 sample at both farms. At both locations antibody titers decreased from d 21 to d 56. Titers decreased in 86% of calves at AZ and in 97% of calves at TX at the anamnestic response (Figure 3.3). The differences in antibody titers from d 21 to d 42, d 21 to d 56, and d 42 to d 56 responses were not normally distributed ( $P < 0.001$ ), as determined by the Shapiro-Wilk Test, so the Kruskal-Wallis Test was used to determine treatment differences. Titers were not found to be significantly different ( $P > 0.05$ ) between reflective and control calves from d 21 to d 42, d 21 to d 56, or for d 42 to d 56 at either location (Table 3.2). When separated by sex at AZ, the difference in antibody titer response was not significantly different ( $P > 0.05$ ) between d 21 to d 42, d 21 to d 56, or for d 42 to d 56 (Table 3.3).

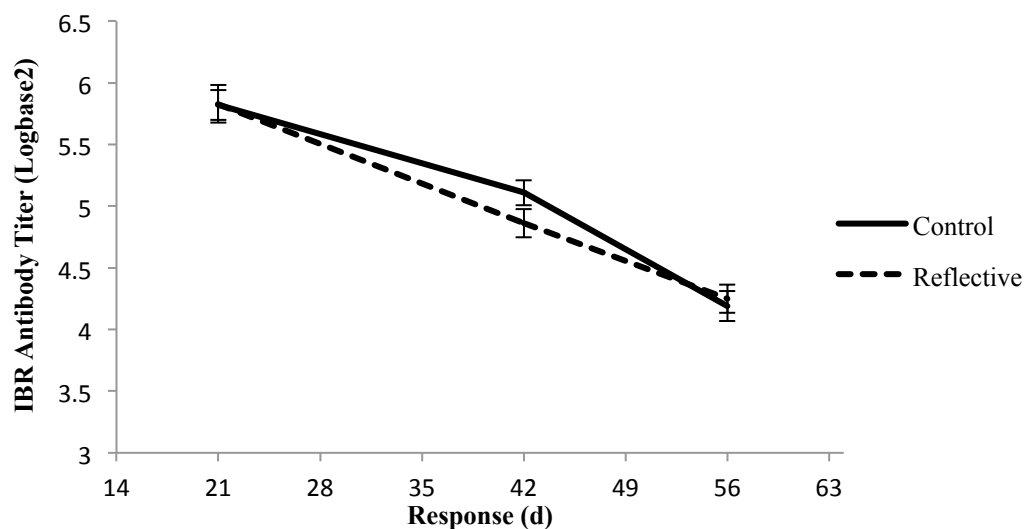


Figure 3.3. Antibody titers of calves housed in reflective and control calves with SE for d 21, d 42, and d 56. Titters were not statistically different ( $P > 0.05$ ). The rate of decline of antibody titers between control (Slope = -0.81) and reflective (Slope = -0.79) were similar.

Table 3.2. Antibody titer differences at different stages of the immune response to IBR vaccination for calves housed in control and reflective hutches.

Farm	Stage (d)	Control (n = 46)	Reflective (n = 36)	p-Value
AZ	21-42	$0.83 \pm 0.15$	$1.02 \pm 0.11$	0.19
TX	21-42	$1.07 \pm 0.16$	$0.86 \pm 0.86$	0.49
AZ	21-56	$1.68 \pm 0.12$	$1.73 \pm 0.14$	0.65
TX	21-56	$2.12 \pm 0.24$	$1.90 \pm 0.21$	0.55
AZ	42-56	$0.77 \pm 0.19$	$0.58 \pm 0.19$	0.18
TX	42-56	$0.74 \pm 0.13$	$0.51 \pm 0.15$	0.25

Table 3.3. Antibody titer differences at different stages to IBR vaccination for male and female calves housed in control and reflective hutches at AZ. For males n = 25 for control and n = 14 for reflective. For females n = 19 for control and n = 22 for reflective.

Stage (d)	Control	Reflective	p-value
Male			
21-42	0.92 ± 0.13	1.07 ± 0.16	0.48
21-56	1.74 ± 0.22	1.67 ± 0.29	0.90
42-56	0.59 ± 0.18	0.42 ± 0.24	0.52
Female			
21-42	0.57 ± 0.17	0.91 ± 0.16	0.22
21-56	2.00 ± 0.22	1.64 ± 0.21	0.56
42-56	0.94 ± 0.20	0.65 ± 0.19	0.28

#### *Weight gain*

At AZ, there was not a significant difference ( $P = 0.14$ ) in weight gain between calves housed in reflective and control hutches for the 56 day trial period (Table 3.4) although a trend in which the reflective calves gained more weight is visible. TX housed calves in the hutches for a 91-day trial period and there was no significant difference ( $P = 0.79$ ) between the calves housed in reflective and control hutches (Table 3.5). Sex of the calves did not significantly influence ( $P > 0.05$ ) weight gain in AZ (Table 3.6).

Table 3.4. Least squares mean for total weight gain (kg) during 56 day trial period for control and reflective housed calves at AZ.

Control Hutches	n	Reflective Hutches	n	p-Value
26.1 ± 1.24	40	29.5 ± 1.59	24	0.14

Table 3.5. Least squares mean for total weight gain (kg) during 91 day trial period for control and reflective housed calves at TX.

Control Hutches	n	Reflective Hutches	n	p Value
53.2 ± 1.67	36	53.9 ± 1.64	37	0.79

Table 3.6. Least squares mean for total weight gain (kg) during 56 day trial period by sex at AZ.

Sex	Control	n	Reflective	n	p-Value
Male	26.8 ± 1.55	25	30.9 ± 1.55	9	0.16
Female	25.7 ± 2.00	15	28.3 ± 2.00	15	0.37

#### *Internal hutch temperature*

Figure 3.4 depicts the internal hutch temperatures between reflective and control hutches during a 24-h period with clear skies. Internal hutch temperature in reflective hutches (n = 5) was 2.16 °C cooler than in control hutches (n = 5) during the hottest 4-h period of the day (P = 0.04) at AZ (Figure 3.5). This period was characterized by little cloud cover. Temperature was also found to be significantly different (P = 0.03) between reflective and control hutches at TX during the hottest 4-h period of the day (Table 3.7). During the coolest 2-h period at night, reflective covered hutches were 0.7 °C warmer than the control (P < 0.05) at AZ. Internal hutch temperature was not found to be significantly different at TX during the coolest 2-h period. Internal hutch

temperature was not found to be significantly ( $P = 0.70$ ) different between Calf-Tel Pro ( $n = 3$ ) and Agri-Plastic EXL ( $n = 5$ ) hutches throughout a 12-h period from 0900 to 2100 (Figure 3.6). But, during the hottest 4-h period of the day, Calf-Tel models showed a trend ( $P = 0.11$ ) to be cooler (Figure 3.6).

Table 3.7. Comparison of mean internal hutch temperature ( $^{\circ}\text{C}$ ) during hottest 4-h period of the day between control and reflective hutches.

Farm	Control ( $^{\circ}\text{C}$ )	n	Reflective ( $^{\circ}\text{C}$ )	n	p-Value
AZ	$42.74 \pm 0.69$	5	$40.58 \pm 0.69$	5	0.04
TX	$37.61 \pm 0.32$	4	$35.05 \pm 0.32$	4	0.03

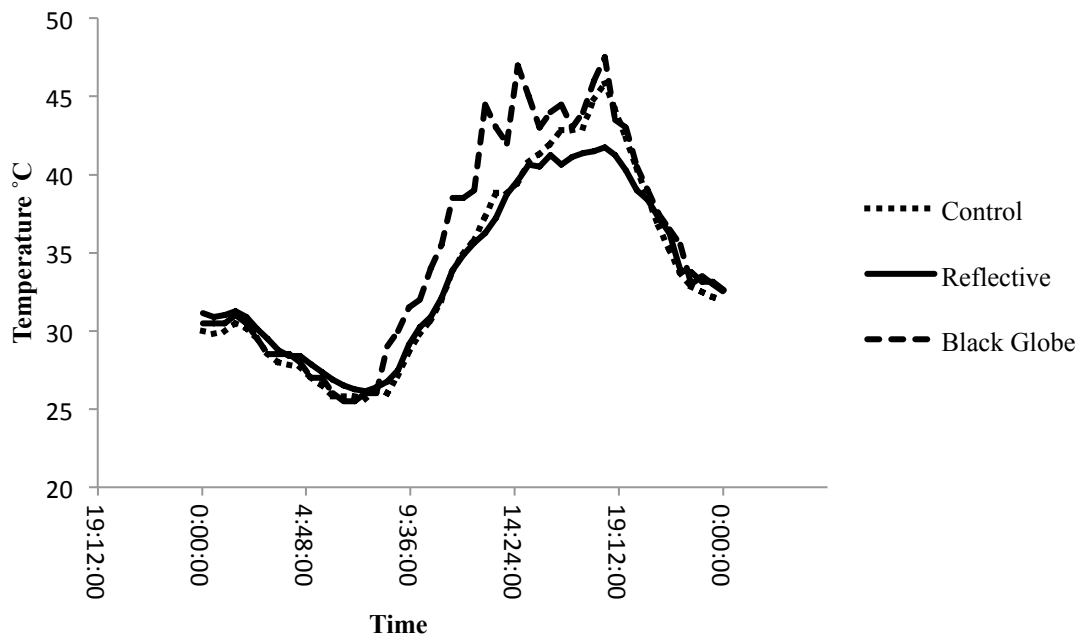


Figure 3.4. Comparison of mean internal hutch temperature during a 24-h period with little cloud cover between reflective and control hutches at AZ. Temperatures were recorded at 30 minute intervals.

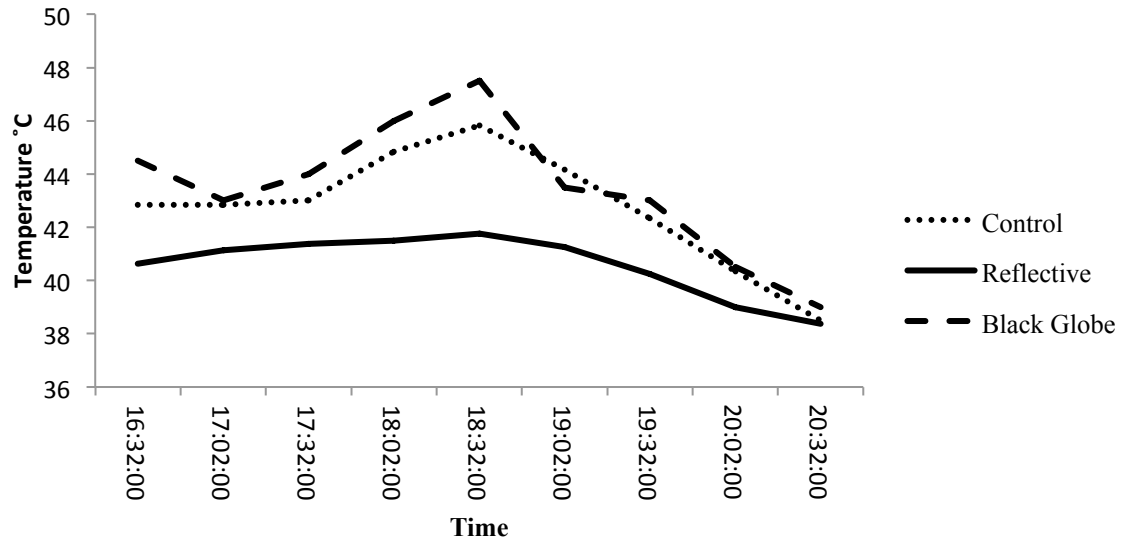


Figure 3.5. Comparison of mean internal hutch temperatures during the hottest 4-hour period of the day between reflective and control hutches at AZ. Temperatures were recorded at 30 minute intervals.

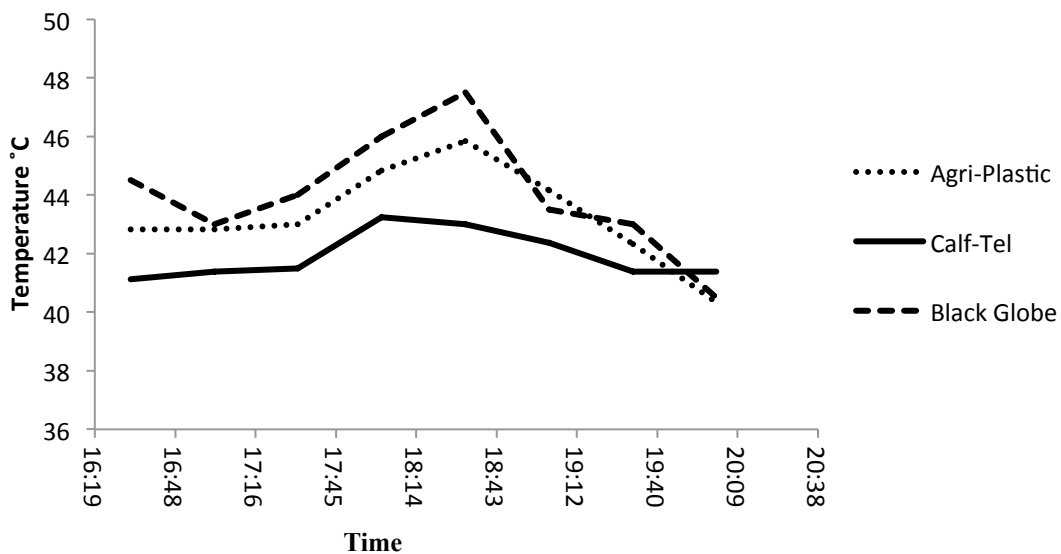


Figure 3.6. Comparison of internal hutch temperature between Agri-Plastic and Calf-Tel hutches during the hottest portion 4-h of the day (1600-2100). Temperatures were recorded at 30 minute intervals.



### *Respiration rates*

Respiration rates were taken on d 21, d 42, and d 56 during the study at AZ. Day 21 and 42 had little to no cloud cover but d 56 was characterized by cloud cover followed by a thunderstorm. Respiration rates were found to have unequal variances ( $P < 0.001$ ) using Brown-Forsythe Test, so Welsh's Test was used to determine treatment effects. Respiration rates for calves housed in reflective hutches were significantly lower ( $P < 0.001$ ) than for control calves during both collection periods with little cloud cover (Table 3.8). Respiration rates were not significantly different ( $P = 0.15$ ) between calves housed in reflective and control hutches on d 56 with cloud cover.

Table 3.8. Mean respiration rate per minute for control and reflectively housed calves with and without cloud cover at AZ.

Collection Day (Presence of Clouds)	Control (bpm)	Reflective (bmp)	p-Value
21 (clear)	72.2 $\pm$ 1.09	61.0 $\pm$ 0.76	< 0.001
42 (clear)	72.8 $\pm$ 0.9	62.4 $\pm$ 1.07	< 0.001
56 (cloudy)	66.2 $\pm$ 1.28	60.6 $\pm$ 1.33	0.15

### *Medical treatments administered*

While housed in the hutches, medical treatment for ear infections at AZ showed a trend ( $P = 0.11$ ) towards calves housed in control hutches receiving more treatments than in reflective hutches, but the total number of retreatments in the same calf was similar (Table 3.9). Calves treated for scours and pneumonia were similar ( $P > 0.05$ ) for calves in control and reflective hutches. Number of repeated treatments for scours and pneumonia were also similar. Death and treatment for pneumonia were recorded for calves and obtained from farm staff at 11 months of age. In the month period following (3-4 months) weaning and calf removal from the hutches, fewer calves that were

reflectively housed received treatment for pneumonia ( $P = 0.01$ ). From 5-11 months or age there was a trend ( $P = 0.09$ ) for more reflective calves to be treated for pneumonia than control calves. There was also a trend for increased survival ( $P = 0.06$ ) of calves housed in reflective hutches as a total of 3 calves died that were housed in control hutches and in reflective hutches from 5-11 months of age. There was no difference in survival between reflectively housed calves and control calves while in the hutch or at 4 months of age. Medical records were not obtained from TX because of the unusual cool summer and unreliable record data.

Table 3.9. Number of calves that received medical treatment and mean number of treatments administered by symptom for calves housed in control and reflective hutches at AZ.

Symptom	Time Period	Number of Cases		
		Control (n = 61)	Reflective (n = 49)	p-Value
Ear Infection	In hutch	13	5	0.11
Pneumonia	In hutch	8	7	0.90
	3-4 months	23	8	0.01
	5-11 months	4	9	0.09
Scours	In hutch	13	11	0.89
Death	5-11 months	3	0	0.06

## **Discussion**

### *Thermal conditions*

Reflective hutch covers work by reflecting radiant energy from direct sunlight away from the hutch. For this reason they are most beneficial during periods with high solar radiation (Friend et al., 2014). High cloud cover and low summer ambient temperatures were prevalent throughout the trial period at TX, while AZ had a “normal” summer of hot and dry conditions, until the start of the monsoon season. The lack of typical hot summer weather in TX led to a separate analysis of data between the two locations.

Although average monthly temperatures maximums exceeded the TNZ at both AZ and TX throughout the study, nightly temperatures were within the calves TNZ, so calves were not under constant heat stress during the study. Nightly average temperature minimums at AZ were 21.1 °C for the month of June (d 0-d 21), 25.6 °C for July (d 21-d 42), and 23.3 °C for August (d 42-d 56). TX nightly average temperature minimums were 17.2 °C for June (d 0-d 21), 17.9 °C for July (d 21-d 42), and 17.6 °C for August (d 42-d 56). Calves may be able to utilize the cool nights to physiologically recover from thermal stress (Silvanikove, 2000).

### *Persistent infection within the herd*

Persistent infection with BVD was not found at either location. Persistent infected calves have a survival rate of 50% within the first 12 months of life and could greatly alter the calf’s immune response (Smith, 2009). Absence of PI individuals indicated that BVD was not a confounder in this study. The absence also is an indicator of good herd health, as PI calves are infected early in gestation after the dam is exposed to BVD. These results can be used to indirectly support the BVD vaccination protocol and biosecurity measures of the farms.

### *Antibody response to IBR vaccination*

Antibody titers were not significantly different between reflective and control calves at either location for the post-colostrum, initial or anamnestic immune response. The most probable explanation for this was the influence of maternal IBR antibodies supplied in the colostrum. All calves, at both locations, received two feedings of pooled colostrum collected from heifers and cows that had been vaccinated against IBR.

Maternal antibodies have a half-life of 21 days (Tizard, 2009). The antibody titer decline from this experiment had a slightly longer half-life than maternal antibodies with a half-life of 33 days. Vaccinated animals may not have increased antibody levels if high levels of maternal antibodies are present (Smith, 2009). Calves at both locations tested positive for IBR antibodies in the post-colostrum sample. Maternal antibodies can prevent the calf from producing its own immune response by inhibiting B cell production. T cell response is much less affected. A proposed explanation for the mechanism of immunosuppression by maternal antibodies is that the maternal antibodies mask the epitopes on the antigens in the IBR vaccine preventing the calf's B cells from recognizing it. Maternal antibody titers must drop below the critical threshold in order for the calf to elicit its own immune response (Tizard, 2009). Even when vaccinated at 84 days of age, calves were unable to seroconvert the IBR vaccination when maternal antibodies were high. But, when revaccinated at 196 days of age, previously vaccinated calves more rapidly seroconverted than single vaccinates (Menanteau-Horta et al., 1985). Vaccination while maternal antibodies are still high may prime the memory cells and be of value. Unfortunately calves are typically housed in individual polyethylene hutches for less than 90 days, so waiting until the calves were older was not possible.

While the high antibody titers to IBR are suspected to have prevented a measurable immune response, they are also an indicator of successful passive immunity being acquired by the calves from the pooled colostrum. This study was able to provide important feedback to the dairies on their colostrum programs.

The similarity in the slope of the titer throughout the study for the control and treatment calves also indicated that the maternal antibodies were declining at a similar rate. If a difference in slope had been observed between treatments, protein catabolism, particularly of IgG1, could be different between control and reflective covered calves.

Although the possibility of maternal antibody interference with the IBR vaccination was considered when planning this experiment, it was not expected that the titers would decrease after both the initial and booster vaccination. The primary immune response to an inactivated vaccine, such as the one used in this study, is composed of primarily IgM antibodies and is fairly weak and short-lived, but can still be detected. The immune response to the booster vaccine was expected to be much stronger and composed of IgG antibodies due to more rapid seroconversion (Tizard, 2009).

The lack of significant difference between antibody titers of control and reflectively housed calves indicates that the use of an antigen, for which the calf has maternal antibodies, cannot be used. Maternal antibody interference can be avoided by using a foreign antigen or by measuring IgA from nasal secretions. While use of a foreign antigen is difficult due to the risk of detriment to the replacement heifer calves, viruses that are used in close-up vaccines such as Coronavirus or Rotavirus, may be a suitable option to limit the potential danger to the calf. IgA serves as the first line of defense at mucosal surfaces and functions independently from maternal immune responses (Kramer and Cebra, 1995; Corthösy, 2007). It may be possible to detect differences in control and reflective covered calves by quantifying IgA in the nasal cavity over a period of prolonged heat, but that data were not collected in this study.

### *Weight gain*

Mean body weight between control and reflective covered calves was not statistically different at either location even when separated by sex at AZ (Table 3.4; Table 3.5). Studies from this laboratory have yet to indicate any influence from the reflective covers on weight gain during hot periods. Calves at both locations were limit-fed pasteurized waste milk twice daily. Calves at AZ were weighed before they began to

consume starter feed. Calves at TX had been transitioned to starter feed approximately two weeks before final weights were collected.

Based on studies with adult dairy cattle, it was expected that reflective covered calves might have a greater mean body weight. Feed intake reduction is one of the first indicators of heat stress in cattle (Collier et al., 1982). The ability to absorb and consume nutrients becomes limited under heat stress conditions and temperature and water consumption increase significantly above 27 °C (Beede and Collier, 1986). Calves in this study regularly consumed their entire ration of milk. It is possible that the heat stress on the calves was not enough to reduce the suckling drive and reduce consumption of the ration. An additional possibility is that because milk is the major component of the calf's diet, the desire of increase liquid consumption was fulfilled by the milk (Funquay, 1981).

It is possible that feeding calves milk ad-libitum might have allowed differences to be detected between reflective and control calves. Differences in voluntary starter consumption and gain after transition from milk while the calves are still in the hutch may be a more sensitive method to determine treatment differences.

#### *Internal hutch temperature*

Reflective covered hutches at AZ were 2.16 °C cooler ( $P < 0.05$ ) than control hutches during the hottest 4-h portion of the day but were 0.7 °C warmer ( $P < 0.05$ ) than the control during the coolest 2-h portion of the night. At TX, reflective covered hutches were 2.56 °C cooler ( $P = 0.03$ ) than control hutches during the hottest 4-h portion of the day, but not significantly different at night. This study indicates that while solar radiation is high, the reflective cover is effective in reducing internal temperature, but while lower, the cover can help retain the hutch's heat. At both AZ and TX nightly temperatures were within the calves' TNZ and calves may have used this time to physiologically recover from the thermal stresses of the day (Silvanikove, 2000). By retaining heat, the covers may slightly reduce the efficiency of this recovery period but during cold temperatures, the heat retaining ability can be beneficial. The data also

indicates the importance of solar radiation on the effectiveness of the reflective hutch covers. Reflective hutch covers are expected to be most effective in reducing internal hutch temperature during high solar radiation, which is when calves are more likely to be at risk of heat stress. Although there was a trend for Calf-tel hutchers to be cooler.

Agri-Plastic and Calf-Tel hutchers' temperatures were not significantly different ( $P = 0.11$ ) during a hot 4-h period or cool 4-h period during the night at AZ. However, the data should be interpreted with caution because of the relatively small number of control Calf-Tel hutchers that were available in AZ. Construction of these specific models of polyethylene calf hutchers was similar, and although not tested for ventilation in this experiment, the Agri-Plastic had a slightly larger back window. It should also be included out that the back ends of all of the hutchers at AZ were elevated 15 cm using concrete blocks, with the goal of maximizing ventilation.

#### *Respiration rates*

Reflective covered calves had significantly lower ( $P < 0.001$ ) respiration rates during periods of no cloud cover at AZ. Rates between control and reflective calves were not significantly different ( $P = 0.15$ ) when there was cloud cover and an impending thunderstorm, highlighting the importance of the solar radiation reflecting qualities of the covers. Internal hutch temperature readings of the surface temperature of the hutchers indicates that during many cloudy days there is still enough radiation present to warm the surfaces of the hutch and reflective hutchers tend to be cooler, but the reduction in temperature is not biologically important.

In cattle, 20 breaths per minute is basal level and at 60-80 breaths per minute, the animal is considered to be indicative of medium to highly heat stress (Silvanikove, 2000). Reflective covered calves had a respiration rate of approximately 10 breaths per minute less than control calves during periods with no cloud cover.

### *Medical treatments*

While in the hutch, fewer reflectively housed calves were treated for ear infections than control calves. Cows under medical treatment, such as antibiotics must be milked separately from the healthy herd. Many dairies prevent milk waste by feeding the hospital bulk tank milk to the calves. Clinical mastitis can result from *Mycoplasma*, which can cause ear infections in the calf if not pasteurized (Walz et al., 1997). The proportion of calves treated for pneumonia in control hutches was significantly higher ( $P = 0.02$ ) than calves in reflective hutches from 3 to 4 months of age but control calves showed a trend ( $P = 0.09$ ) for fewer treatments from 5-11 months of age. Number of treatments per calf for pneumonia was similar between control and reflective calves. Calves were removed from the hutch at around 90 days of age and were placed into pens in groups of 10. It is possible that the reflective covers gave the calves an advantage over control calves during the stressful period of weaning and transition to group housing. From 5-11 months of age medical records only indicated treatment for pneumonia and not for other illness. It is possible that the trend toward fewer control calves receiving medical treatment for pneumonia from 5-11 months of age may have been influenced by the change in medical recording and should be examined further.

This is important because heat stress in utero has been documented to have long term effects on the calf, including lowered milk production at first lactation (Monteiro, 2013).

### **Implications**

Reflective hutch covers are most effective in reducing the internal hutch temperature of polyethylene calf hutches during high ambient temperature and low cloud cover. Under these conditions reflective covers reduced respiration rates of calves, but did not affect weight gain. Due to interference with maternal antibodies, the use of an IBR vaccination was unable to detect the cover's effect on immunologic response. Reflective covered calves required less medical treatments than control calves while housed in the hutches. The number of calves treated for pneumonia from reflective



covered hutches was also lower throughout the first 11 months of life, indicating not only the long range benefits of the covers, but justifying the cost of the cover as well.

Total consumption of milk was observed in both control and reflective covered calves. It was unclear whether calves were not experiencing appetite suppression in response to the high ambient temperatures, or if calves were continuous to consume milk because of a need to by increasing their fluid intake. However the calves did have access to water. Future studies can better detect the effects of the reflective covers on milk consumption and weight gain while pre-weaned calves are fed milk ad-libitum.

Using a foreign antigen or the measurement of IgA can be more effective in determining the immunological effects of the reflective hutch covers during high ambient temperature. Both methods would minimize interference with maternal antibodies in order to detect treatment differences. However convincing commercial dairy farmers to agree to vaccinate their heifer calves with foreign antigens is problematic.

This study also indirectly provided a survey of the farms' vaccination, biosecurity, and colostrum programs. Both being large dairies (>500 cows), BVD can be a very expensive issue effecting reproduction and calf health. The absence of PI's can provide some indication that biosecurity and vaccination programs against BVD are effective. The effectiveness of the farms' colostrum programs was indicated by the high antibody titers to IBR after the calf had received colostrum. Colostrum is a vital start to the calf's health and is particularly important during heat stress.

## **CHAPTER IV**

### **USING REFLECTIVE HUTCH COVERS TO REDUCE COLD STRESS ON CALVES IN POLYETHYLENE HUTCHES**

#### **Introduction**

Holstein cattle are temperate weather animals and performance begins to decline as THI exceeds 68 in high producing cows (Collier et al., 2006). While cows can be expected to perform better in winter than summer months, cold weather presents additional challenges for the neonate. Neonates do not have the metabolic heat producing ability that adults do and are therefore more sensitive to cold temperatures. Cold calves must divert energy from growth to maintain body temperature. Weight is the primary measurement for production value in heifer calves due to the negative correlation of body weight and onset of puberty (MacDonald et al., 2005). Heavier heifer calves enter the production cycle earlier and reduce its capital investment (Bungert, 1998). Bedding and calf jackets can be used to prevent cold stress, but during periods of precipitation, both bedding and calf jackets can cause the calf to rapidly lose body heat. The increased density of water allows the bedding and jackets to conduct heat away from the body 25 times faster than air alone. In order to prevent rapid heat loss, a labor force must frequently monitor the calves and the weather. An alternate solution is to heat the hutch itself.

Previous research by this lab has suggested that reflective covers may maintain the microclimate of the hutch at a higher temperature during cool periods at night than control hutches (Friend et al., 2014; Binion et al., 2015). Binion et al. (2015) also found that on cold sunny days the internal hutch temperature was reduced by the reflective hutch cover by blocking direct radiation onto the hutch from the sun.

The objective of this study was to determine if the LDPE cover blocked too much sun during the day for its heat retaining abilities during the night, to be beneficial. This study aimed to validate the use of LDPE covers on a commercial dairy setting

during winter months to improve calf welfare. It can be inferred that calves who spend less energy maintaining their body temperature can allocate more energy to growth and are more comfortable.

## **Materials and methods**

The study was conducted on a large commercial dairy (6,000 cows) near Plainview, TX during two consecutive winters (Trial 1 and Trial 2). Animal care and use was approved by the Institutional Animal Care and Use Committee of Texas A&M University (AUP # 2012-205A). Both trials were conducted from the first week of December to the third week of February, 2014 and 2015. Calendar months December, January, and February will be used for the ease of explanation of climatic data for the study period. Holstein, Jersey, and Holstein-Jersey cross heifers were used in this study. Calves were removed from the dam at birth and housed in an enclosed nursery. Heifers received two feedings of pooled colostrum via stomach tube within the first 5 hours of birth. One feeding was within an hour of birth and the second within 4 hours of birth. At 2 days of age, heifers were transferred from the nursery into individual polyethylene hutches, Calf-tel Pro (Calf-Tel Pro, Hampel Corp., Germantown, WI, USA) and Calf-Tel Pro II (Calf-Tel Pro II, Hampel Corp., Germantown, WI, USA). Trial 1 used only Calf-Tel Pro and Trial 2 used both Calf-Tel Pro and Calf-Tel Pro II. Hutches were situated on a slight incline so any drainage would move from the hutch opening out the back of the hutch. Both farms utilized a 1.2 x 1.8m outdoor wire pen attached to the hutch that was made from a cattle panel. Calves remained under the farms' protocol for feed, water, and medical care.

Hutches alternated amongst three treatment types: reflective, non-reflective, and the control. The reflective cover consisted of 3.0 mil black LDPE with an aluminized side which faced the hutch. The non-reflective cover was 4.0 mil black LDPE. The covers were installed on the outside of the hutch covering the top, sides and back of the hutch. The covers were approximately 1.8 x 3 m and had a 0.13 m sleeve along its 1.8 m sides. They were secured with a 1.85 m long Schedule 20 polyvinyl chloride (PVC) pipe

that was inserted into its side sleeves. The ends of PVC pipe were attached to 2 custom bungee cords with hooks that attached underneath the hutch to anchor it. The cover draped down the back of the hutch and had a pocket that ran across the bottom of the back that was filled with sand to reduce the ability of the wind to remove or alter the fit of the covers. The installed hutch covers are shown in Figure 4.1. The covers were adjusted occasionally through the study for proper fit on the hutch.



Figure 4.1. Installed reflective (left) and non-reflective (right) covers on South facing Model 1 hutches.

Trial 1 used all Calf-tel Pro (Model 1) hutches with a total of 20 reflective, 10 non-reflective, and 40 control hutches. Trial 2 used both Calf-Tel Pro (Model 1) and Calf-Tel Pro II (Model 2) and a total of 40 reflective covers on Model 1 hutches; 10 non-

reflective covers on Model 1 hutches and 10 on Model 2 hutches; and 60 control hutches (50 Model 1 hutches and 10 Model 2 hutches).

#### *Internal hutch temperature*

Similar to the heat stress study, iButtons (iButton, model 1921G, Maxim Integrated Products, Sunnyvale, CA, USA) were used to collect temperature in a sample of both the control and treatment hutches. The iButtons were allowed to run for approximately 20 minutes then readings were compared to ensure the temperature recordings were within 0.8 °C of each other. Calibrated iButtons were mounted at calf level when lying (0.3 m above the ground). The iButtons were placed in a 1.8 cm wide groove cut into a piece of foam insulation. The foam served to insulate the iButton from the wall of the hutch. Wire mesh was used to cover the insulated iButtons to prevent the calf from tampering with it. The insulated and protected iButtons were fastened to the inside of the hutch as described in Chapter III. Radiant heat was recorded by placing a calibrated iButton into a flat-black-painted-table tennis ball. Black electrical tape was used to seal the iButton in the ball. Two painted table tennis balls were placed in full sun attached to a wooden dowel. Ambient temperature was recorded by placing a calibrated iButton under a completely shaded feed bunk. The iButtons under the feed bunk were mounted in the foam block as described above. The iButtons were secured to the feed bunk using duct tape. The iButtons recorded temperature every 30 minutes and allowed temperature difference to be determined between treatments. Treatment differences were also determined between Model 1 and Model 2 hutches.

#### *Weight gain*

Weight was measured for each calf at birth and then at day 85 for Trial 1 and day 84 for Trial 2 by farm staff. Weight gain of control calves was compared to calves housed in reflective and non-reflective hutches.

### *Medical treatment*

Medical records for calf treated with antibiotics were obtained from the farm upon removal of calves from the hutches for both trials. Medical records were used to ensure that illness did not influence weight gain. Medical records were not detailed enough to compare treatment of illness between control and covered calves.

### *Weather data*

Weather data was obtained from the Weather Underground website ([www.wunderground.com](http://www.wunderground.com)) which sourced the weather from the Hale County Airport.

### *Statistical analysis*

Treatment differences in weight gain between control, reflective, and non-reflective covered calves were determined using ANOVA followed by LSD. Weights are reported as the least squares mean with the standard error of the mean.

In Trial 1, iButtons were fitted in five Model 1 control hutches, five Model 1 reflective hutches, and five Model 1 non-reflective hutches. In Trial 2 the iButtons were placed in five Model 1 control hutches, three Model 2 control hutches, five Model 1 reflective hutches, three Model 1 non-reflective hutches, and three Model 2 non-reflective hutches. Both Trials were split into two periods for temperature analysis: the first period included week 1 of December through week 2 of January and the second period included week 3 of January through week 3 of February. Readings from duplicate iButtons from each hutch were averaged. The same procedure was used for ambient and black globe iButtons. Average temperatures for control, reflective, non-reflective, black globe and ambient iButtons were analyzed using ANOVA, followed by LSD. The data were assessed for normality of distribution using the Shapiro-Wilks Test and included the removal of outliers. It was also assessed for equal variances using the Brown-Forsythe Test. Standard error of the mean is presented with each mean unless otherwise indicated.

## Results

The study period included the first week of December through the third week of February and the calendar months of the study period will be used for ease of explanation of climactic data. The temperature lows during December and January were colder ( $P < 0.01$ ) during Trial 1 than Trial 2. There was not a significant difference in temperature between February in Trial 1 and February in Trial 2. The average low temperatures of the trial periods are summarized in Table 4.1.

Table 4.1. Average minimum temperature for each month between Trial 1 and Trial 2. Minimum monthly temperatures for December and January were significantly different ( $P < 0.001$ ) between Trial 1 and Trial 2.

Trial	Month					
	December		January		February	
	1	2	1	2	1	2
Temperature (°C)	-5.00	2.62	-6.18	2.88	-3.29	-3.07
Temperature Difference (°C)	2.62 <sup>a</sup>		2.88 <sup>a</sup>		0.21	

<sup>a</sup> Difference is significantly different ( $P < 0.01$ )

### *Internal hutch temperature*

When 30 minute intervals of nightly temperature was averaged over a 7-day period, selected because of clear skies from 2130-0530 during Trial 1, reflective hutches were  $1.22 \pm 0.35$  °C warmer ( $P < 0.001$ ) than control hutches in Trial 1 and non-reflective hutches were warmer ( $P < 0.05$ ) by  $0.67 \pm 0.35$  °C (Figure 4.1). Nightly internal temperature was also greater ( $P < 0.01$ ) in reflective and non-reflective ( $P < 0.05$ ) hutches than in control hutches during a similar 7-day period with clear skies from 2130-0530 during Trial 2. Reflective hutches also showed a trend towards cooling down at a slower rate than control hutches as the sun set (1720-1920). Reflective hutches were also  $1.53 \pm 0.86$  °C warmer ( $P = 0.07$ ) in Trial 1 and  $1.65 \pm 0.85$  °C warmer ( $P = 0.06$ ) in Trial 2 as the sun set (1720-1920). The temperature difference between control and non-reflective hutches was not significant at this time period.

When a 2-h period of morning sun with clear skies was identified over 6 days (0800-1000) during Trial 1, reflective hutches were warmer ( $P = 0.01$ ) than control hutches by  $1.5 \pm 0.60$  °C. Non-reflective hutches were warmer than the control hutches by  $1.0 \pm 0.60$  °C but not statistically different ( $P = 0.08$ ). This trend was also found in Trial 2 (Figure 4.3) in which reflective hutches were warmer ( $P < 0.01$ ) than controls by  $1.29 \pm 0.32$  °C, but non-reflective covers were not significantly warmer  $0.93 \pm 0.32$  °C ( $P = 0.09$ ).

Internal hutch temperature was not significantly different ( $P > 0.05$ ) during the day (0800-2000) when 30 minute intervals were averaged over the entirety of each trial period, even when sunny, clear days were identified.

Model 1 and Model 2 control hutches were not different ( $P > 0.05$ ) throughout Trial 2 during periods of clear skies. Figure 4.4 illustrates the internal hutch temperature between Model 1 and Model 2 hutches for a 12-h period during the day. There was also not a significant difference ( $P > 0.05$ ) between the internal hutch temperature of reflective and non-reflective hutches.



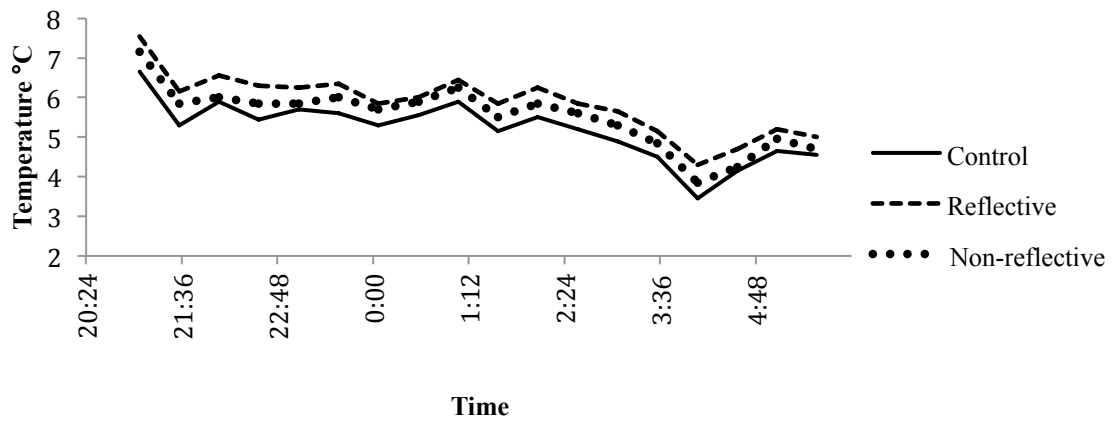


Figure 4.2. Average internal hatch temperature of control, reflective, and non-reflective hatches from 2130-0530 over 7 periods with clear skies in Trial 1. Temperatures were recorded at 30 minute intervals.

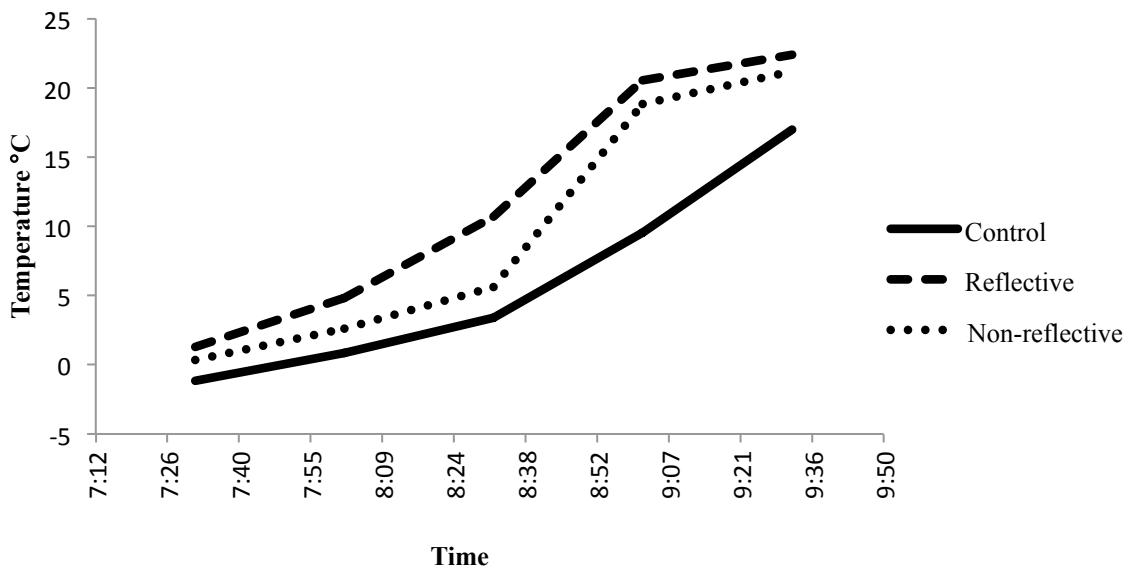


Figure 4.3. Average internal hatch temperature of control, reflective, and non-reflective hatches from 0730-0930 over 7 periods with clear skies in Trial 2. Temperatures were recorded at 30 minute intervals.

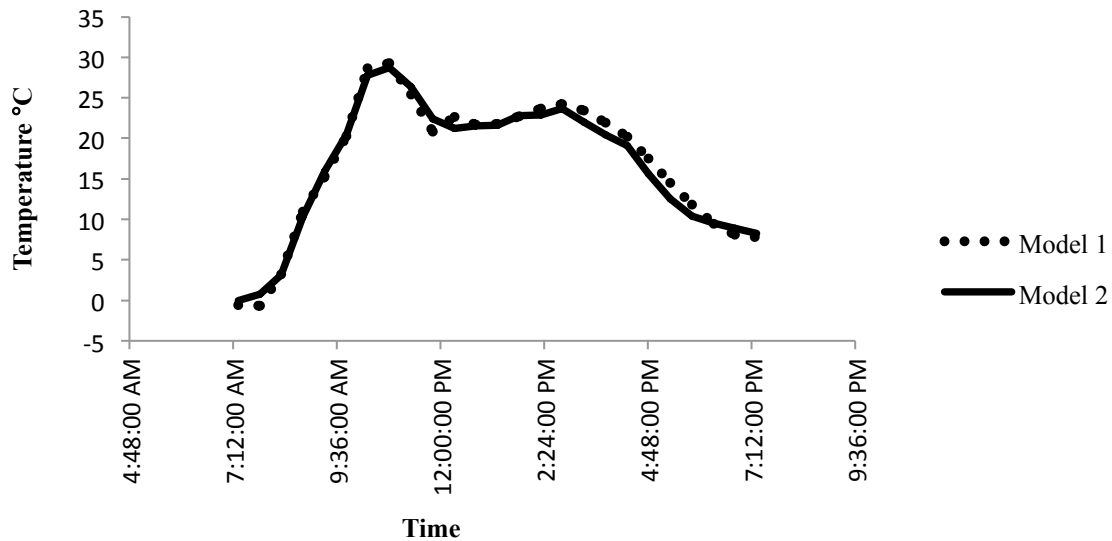


Figure 4.4. Internal hutch temperature of Model 1 and Model 2 hutches from 0700-1900 averaged over 7 days with clear skies during Trial 2. Temperatures were recorded at 30 minute intervals.

### *Weight gain*

Calves housed in non-reflective hutches had a greater ADG than calves housed in control hutches during Trial 1 ( $P < 0.05$ ), but the difference in ADG was not higher during Trial 2 ( $P > 0.05$ ) for calves housed in Model 1 hutches. Reflective covered calves did not have a significantly higher ( $P > 0.05$ ) ADG in either trial.

When comparing control and non-reflectively covered calves between Model 1 and Model 2 hutches, differences in ADG were not statistically different ( $P > 0.05$ ).

Table 4.2. Comparison of ADG in kg for calves housed in Model 1 hutches for Trial 1 and Trial 2.

Trial	Control		Reflective		Non-reflective	
	1	2	1	2	1	2
n	40	50	20	40	10	10
Age (d)	84	85	84	85	84	85
ADG (kg)	0.66 <sup>a</sup>	0.65	0.70	0.61	0.72 <sup>a</sup>	0.68

<sup>a</sup> significantly different within each trial (P < 0.05)

Table 4.3. Comparison of ADG between Model 1 and Model 2 hutches for control and non-reflective calves in Trial 2.

Hutch Model	Treatment	n	Age (days)	ADG (kg)
1	Control	50	84	0.65 ± 0.02
2	Control	10	84	0.64 ± 0.02
1	Reflective	40	84	0.61 ± 0.02
1	Non-reflective	10	84	0.68 ± 0.02
2	Non-reflective	5	84	0.59 ± 0.04

## Discussion

Although average minimum temperatures were statistically different between Trial 1 and 2 for December and January, the average minimum temperature during both trials were below the lower critical temperature for dairy calves (0 °C). The low temperature would suggest that during both trials the calves were subject to cold stress.

### *Internal hutch temperature*

In agreement with previous studies by this lab (Carter et al., 2012; Binion et al., 2014; Friend et al., 2014), hutch covers again demonstrated heat retaining abilities during low ambient temperatures. Reflective and non-reflective covers kept internal hutch temperature higher during the night and reflective covers delayed the decrease in temperature as solar radiation diminished. Binion et al, (2015) suggested that the air space located between the hutch cover and the hutch would prevent the hutch from heating up as much as the control hutches during sunny periods. This study found that although internal hutch temperature was not significantly different during the day, the reflective hutches heated up faster during a 2-h of morning sun with clear skies over a 6-day period during both trials.

Although there was not a significant difference in internal hutch temperature between Model 1 and Model 2, hutches both the reflective and non-reflective hutch covers were designed to fit on Model 1 hutches. Model 2 hutches had a slanted back that prevented a taunt fit of the cover onto the hutch, making it more susceptible to flapping and removal by high winds. Out of the 10 non-reflective hutch covers applied to Model 2 hutches, 5 blew off throughout the study, reducing the sample size for weight gain of the non-reflective covered calves

### *Weight gain*

As a result of the wind removing several of the non-reflective hutch covers, the number of calves used for the calculation of ADG was reduced by half. It is possible that the small sample size of 5 did not accurately represent the abilities of the non-reflective cover based on the higher ADG from the calves housed in the Model 1 hutches from both Trial 1 and Trial 2. ADG of large breed calves is 0.77 kg/day and 0.59 kg/day for small breed calves (Hopkins and Witlow, 2013). The majority of calves were Holstein/Jersey crosses and the average daily gain of the calves involved in this study were in between these values.

Replacement heifers account for approximately 30% of the feed costs for the farm with the pre-weaning period being the most costly. Higher maintenance requirements during winter amplify this cost, bringing the necessity of cold abatement techniques to light (Eastridge, 2014). By lowering the calf's maintenance requirements, more energy from feed could be utilized for growth. Below the calf's TNZ, ADG is decreased without nutritional supplementation (Scibilia et al., 1987). Calves in this study were fed on a fixed ration of milk and a difference in weight gain was only observed during Trial 1 with the non-reflective covers. Additional nutrition can not only support the calf's increased maintenance requirements during inclement weather, but ad-libitum feeding can also increase weight before weaning and maintained weight advantage until 2 months of age (Jasper and Weary, 2002). It is possible that a difference in ADG could be determined by feeding milk ad libitum or if calves had been fed starter feed for a longer period while housed inside the hutch.

The significantly colder temperature lows in December and January of Trial 1 may account for the reason non-reflectively covered calves had significantly higher ADG in Trial 1 but not in Trial 2. Although temperature lows were below the TNZ of the calves for both trials, energy savings from the hutch covers may be more apparent and detectable when weather is colder than during this study.

It is not clear why there was an observable difference in ADG between non-reflective covered calves but not with the reflective. Non-reflective covers were 4.0 mil thick, whereas, reflective covers were 3.0 mil. Non-reflective covers were found to be slightly warmer during the night than reflective covers but when the sun was shining, reflective covers were slightly warmer. This may be because the sample size for the non-reflective covers was small. The apparent difference between day and nighttime temperatures may balance out the advantages between the covers. A significant difference was not observed between the two hutch cover types, but weight gain was nominally higher in Trial 2 as well. It is possible the difference in ADG may be a statistical artifact attributed to random variation among the calves. More research is necessary to distinguish between the importance of the aluminized surface reflecting

heat back into the hutch versus the additional thickness of the non-reflective covers. This would best be analyzed in a controlled environment without the variables of wind, cloud, cover, and precipitation as seen in this experiment.

### **Implications**

Although the use of reflective and non-reflective hutch covers has been found to influence internal hutch temperature, evidence of the influence on ADG from this study was inconclusive. A better measure of ADG may be determined by holding calves in the hutches for a longer period while they are on ad-libitum feed. The calves in this study were limit-fed and regularly consumed the entire ration of feed. Nightly internal hutch temperature was increased in both trials by the use of the reflective covers, but daytime temperature was not significantly different between either cover, and the control hutches. It is possible that the heat retaining ability of the cover has more effect during cold weather than the cover's ability to absorb heat from the sun during direct solar radiation and transfer the heat to the hutch.

Further research is necessary to determine if the cost of purchasing the hutch covers, which although is fairly inexpensive, and the labor to install and maintain the covers can be justified by increased ADG or other biologically significant parameters.

## **CHAPTER V**

### **GENERAL CONCLUSIONS**

To improve the welfare of production animals, recommendations must be cost effective and practical. To ensure the practicality of the use of LDPE covers on reducing thermal stress in dairy calves, the hutch covers needed to be tested in large volumes during the winter and summer months. Unfortunately this also leaves these studies at complete mercy of the environment. The variability of the environment did not always generate conditions that were ideal for the study of heat and cold stress during the study, but several general conclusions can still be made regarding the effectiveness of the LDPE individual hutch covers.

#### **Environmental stress**

Heat and cold stress is well described in the adult dairy cow, particularly its effects on lactation, DMI, and reproduction. Abatement techniques for inclement weather are largely tailored for use on the lactating animal, leaving much room for improvement in calf management. As dairies continue to expand throughout the Southwest United States, annual exposure to temperature outside the TNZ increases. Temperatures outside the TNZ cause energy diversion from growth and body function to thermoregulation. Humidity, wind, direct solar radiation, and precipitation can instigate this energy diversion. Replacement heifers account for approximately 30% of the feed costs for the farm and by decreasing ADG, inclement weather causes calves to take longer to reach maturity, delaying calving and returns from milk production (Bungert, 1998).

Several findings from these studies suggest the effectiveness of LDPE hutch covers in moderating the internal hutch temperature of individual polyethylene hutches. During high ambient temperature and direct solar radiation, reflective covered hutches were cooler than uncovered hutches. Calves housed in reflective covered hutches had

lower respiration rates and a lower number of calves were treated for pneumonia than calves in uncovered hutches, indicating the possible biological significance of the covers on a short-term and long-term basis. During low ambient temperatures, covered hutches retained heat inside the hutch and kept them warmer during the night; also showing a trend toward not only cooling down faster after sunset, but also heating up faster during the first hours of sunlight.

This study also provided valuable information from indirect observation of the farms' protocol against BVD infection and colostrum program. By assessing status of PI's and antibody titers to IBR, both farms were concluded to have had effective practices based on our sample size.

### **Future investigation**

Although environmental conditions varied throughout the study and were not always stressful enough to induce a physiological response, these studies provide valuable information in determining the practicality of LDPE covers on a commercial dairy. In comparison to the lactating animal, information on the effects of heat and cold stress on biological function is lacking in the dairy calf. Identification of specific temperature thresholds on growth and immunity are of particular necessity. While this study aimed to validate the use of the covers using the biological function of the calves, an artificial environment with completely controlled settings would not accurately test the covers for use on the constantly changing environment of a commercial dairy. A suitable material for the hutch covers has been under investigation by this laboratory for many years. Therefore, not only was the effect of the hutch cover on internal hutch temperature important, but the longevity of the material as well.

The utilization of LDPE hutch covers was successful at moderating the internal hutch temperature of polyethylene hutches in both low and high ambient temperatures. Hutch covers offer a realistic approach to managing temperature extremes because they can be manufactured at a relatively low cost (under \$5.00) for a variety of hutch models and require little labor for maintenance.



Future research is necessary to effectively validate the effect of LDPE covers on the biological function of calves including weight gain and immune function. LDPE covers did not affect weight gain conclusively in this study, but use of calves fed ad-libitum, may allow a discernable difference to be detected between calves in covered and uncovered hutches. Calves in these studies were limit-fed and consumed their entire ration during feeding. Additional nutrition can not only support the calf's increased maintenance requirements during inclement weather, but ad-libitum feeding can also increase weight before weaning and maintained weight advantage until 2 months of age (Jasper and Weary, 2002). Due to interference with maternal antibodies, the IBR vaccination protocol was not an efficient method in measuring biological function. Future studies may be able to detect differences in immunological function by immunization with a foreign antigen or measurement of IgA in nasal cavities.

## LITERATURE CITED

- Armstrong, D. V. 1994. Heat stress interaction with shade and cooling. *J. Dairy Sci.* 77:2044-2050.
- Azzam, S.M., J.E. Kinder, M.K. Nielsen, L.A. Werth, K.E. Gregory, L.V. Cundiff, and R.M. Koch. 1993. Environmental effects on neonatal mortality of beef calves. *J. Anim. Sci.* 71:282–290.
- Beam, A.L., J.E. Lombard, C.A. Koprak, L.P. Garber, A.L. Winter, J.A. Hicks, and J.L. Schlater. 2009. Prevalence of failure of passive transfer of immunity in newborn heifer calves and associated management practices on US dairy operations. *J. of Dairy Sci.* 92:3973–3980.
- Beede, D. K., and R. J. Collier. 1986. Potential nutritional strategies for intensively managed cattle during thermal stress. *J. Anim Sci.* 62: 543-554.
- Berman, A. 2005. Estimates of heat stress relief needs for Holstein dairy cows. *J. Anim. Sci.* 83: 1377-1384.
- Bianca, W. 1976. The significance of meteorology in animal production. *Int. J. of Biometeorology* 20:139-156.
- Binion, W. R., T. H. Friend, and G. A. Holub. 2014. Usefulness of an aluminized polyester film for reducing heat in polyethylene calf hutches. *Int J Biometeorol.* 58:1819-23.
- Binion, W.R., and T.H. Friend. 2015. Modeling the effect of reflective calf hutch covers on reducing heat loss. *Int J Biometeorol.* 1–3.
- Brouček, J., M. Letkovičová, and K. Kovalčuj. 1991. Estimation of cold stress effect on dairy cows. *Int J Biometeorol.* 35:29–32.
- Bungert, K. 1998. Calves feel the heat, too. *Dairy Herd Management.* 35:15.
- Carter, B. H., T. H. Friend, S. M. Garey, J. A. Sawyer, M. B. Alexander, and M.A. Tomaszewski. 2012. Efficacy of reflective insulation in reducing heat stress on dairy calves housed in polyethylene calf hutches. *Int J Biometeorol.* 58:51-59.
- Charkoudian, N. 2010. Mechanisms and modifiers of reflex induced cutaneous vasodilation and vasoconstriction in humans. *J Appl Physiol* (1985). 109:1221–1228.

- Chase, C.C.L., D.J. Hurley, and A.J. Reber. 2008. Neonatal Immune Development in the Calf and Its Impact on Vaccine Response. *Vet. Clin. North Am.: Food Anim. Pract.* 24:87–104.
- Coleman, D. A., B. R. Moss, and T. A. McCaskey. 1996. Supplemental shade for dairy calves reared in commercial calf hutches in a southern climate. *J.Dairy Sci.* 79:2038-2043.
- Collier, R. J., D. K. Beede, W. W. Thatcher, L. A. Israel, and C. J. Wilcox. 1982. Influences of environment and its modification on dairy animal health and production. *J. Dairy Sci.* 65: 2213-2227.
- Collier, R. J., G. E. Dahl, and M. J. VanBaale. 2006. Major advances associated with environmental effects on dairy cattle. *J. Dairy Sci.* 89: 1244-1253.
- Corthösy, B. 2007. Roundtrip ticket for secretory IgA: role in mucosal homeostasis? *J. Immunol.* 178:27–32.
- Drackley, J. K. 2005. Early growth effects on subsequent health and performance of dairy calves. Pages 213-235 in *Calf and Heifer Rearing*. P.C. Garnsworthy, ed. Nottingham Univ. Press, Nottingham, UK.
- Dreiling, C. E., F. S. Carman 3rd, and D. E. Brown. 1991. Maternal endocrine and fetal metabolic responses to heat stress. *J. Dairy Sci.* 74:312–327.
- Eastridge, M.L. 2014. Managing Dairy Calves and Heifers during the Winter Months - extension. <http://www.extension.org/pages/65903/managing-dairy-calves-and-heifers-during-the-winter-months#.VWO6GbnBwXA> Accessed May 25, 2015.
- Ellis, J., S. Gow, K. West, C. Waldner, C. Rhodes, G. Mutwiri, and H. Rosenberg. 2007. Response of calves to challenge exposure with virulent bovine respiratory syncytial virus following intranasal administration of vaccines formulated for parenteral administration. *J. Am. Vet. Med. Asso.* 230:233–243.
- Finch, V. A. 1985. Comparison of non-evaporative heat transfer in different cattle breeds. *Aust. J. Agric. Res.* 36: 497-508.
- Frank, J.W., J.A. Carroll, G. L. Allee, and M.E. Zanelli. 2003. The effects of thermal environment and spray-dried plasma on the acute-phase response of pigs challenged with polysaccharide. *J. of Anim. Sci.* 81:1166-1176.

- Friend, T. H., J. A. Haberman, and W. R. Binion. 2014. Effect of four different reflective barriers on black-globe temperatures in calf hutches. *Int. J. Biometeorol.* 58:2165-2168
- Fuquay, J. W. 1981. Heat stress as it affects animal production. *J. Anim. Sci.* 52: 164-174.
- Godden, S., and R. Wallace. 2004. Health management for dairy calves. *J. of Dairy Sci.* 87:1961.
- Godden, S.M., D.M. Haines, K. Konkol, and J. Peterson. 2009. Improving passive transfer of immunoglobulins in calves. II: Interaction between feeding method and volume of colostrum fed. *J. of Dairy Sci.* 92:1758–1764.
- Graham, S. 1999. NASA Earth Observatory :Clouds & Radiation. <http://earthobservatory.nasa.gov/Features/Clouds/> Assessed May 25, 2015.
- Hahn, G. L. 1999. Dynamic responses of cattle to thermal heat loads. *J. Anim. Sci.* 77: 10-20.
- Hales, J. R. S., C. Jessen, A. A. Fawcett, and R. B. King. 1985. Skin area and capillary dilatation and constriction induced by local skin heating. *Pflugers. Arch.* 404: 203-207.
- Hanon, J.-B., Y. Stede, A. Antonissen, C. Mullender, M. Tignon, T. den Berg, and B. Caij. 2014. Distinction between persistent and transient infection in a bovine viral diarrhoea ( BVD) control programme: appropriate interpretation of real-time RT- PCR and antigen- ELISA test results. *Transboundary & Emerging Diseases.* 61:156–162.
- Hill, T. M., H. G. Bateman, II, J. M. Aldrich, and R. L. Schlotterbeck. 2011. Comparisons of housing, bedding, and cooling options for dairy calves. *J. Dairy Sci.* 94:2138-2146.
- Hopkins, B. A., and L.W. Whitlow. 2013. Feeding dairy heifers from weaning to calving. NCSU ANS 01–203D. North Carolina State University Extension Bulletin, Raleigh.
- Jasper, J., and D.M. Weary. 2002. Effects of Ad Libitum Milk Intake on Dairy Calves. *J. of Dairy Sci.* 85:3054–3058.
- Jorgensen, L. J., N. A. Jorgensen, D. J. Schingoethe, and M. J. Owens, M. 1970. Indoor versus outdoor calf rearing at three weaning ages. *J. Dairy Sci.*, 53:813--816.

- Kramer, D.R., and J.J. Cebra. 1995. Role of maternal antibody in the induction of virus specific and bystander IgA responses in Peyer's patches of suckling mice. *Int. Immunol.* 7:911–918.
- Lance, S. E., Miller, G. Y., Hancock, D., Bartlett, P. C., Heider, L. E., & Moeschberger, M. L. 1992. Effects of environment and management on mortality in preweaned dairy calves. *J. Am. Vet. Med. Assoc.* 201:1197-1202.
- MacDonald, K.A., J.W. Penno, A.M. Bryant, and J.R. Roche. 2005. Effect of feeding level pre- and post-puberty and body weight at first calving on growth, milk production, and fertility in grazing dairy cows. *J. of Dairy Sci.* 88:3363–3375.
- McDowell, R. E. 1972. *Improvement of Livestock Production in Warm Climates.* W. H. Freeman & Co, San Francisco.
- McGuirk, S.M., and M. Collins. 2004. Managing the production, storage, and delivery of colostrum. *Vet. Clin. North Am.: Food Anim. Pract.* 20:593–603.
- McGuirk, S. M. 2008. Disease management of dairy calves and heifers. *Vet. Clin. North Am.: Food Anim. Pract.* 24: 139-153.
- Mellado, M., E. Lopez, F.G. Veliz, M.A. De Santiago, U. Macias-Cruz, L. Avendaño-Reyes, and J.E. Garcia. 2014. Factors associated with neonatal dairy calf mortality in a hot-arid environment. *Livest. Sci.* 159:149–155.
- Menanteau-Horta, A.M., T.R. Ames, D.W. Johnson, and J.C. Meiske. 1985. Effect of maternal antibody upon vaccination with infectious bovine rhinotracheitis and bovine virus diarrhea vaccines. *Can J Comp Med.* 49:10–14.
- Monteiro, A. P. 2013. Impact of maternal heat stress during late gestation on calf performance and health. MS Thesis. Univ. of Florida.
- Moore, D. A., J. L. Duprau, and J. R. Wenz. 2012. Effects of dairy calf hutch elevation on heat reduction, carbon dioxide concentration, air circulation, and respiratory rates. *J. Dairy Sci.* 95:4050-4054.
- National Research Council. 2001. *Nutrient requirements of dairy cattle.* 7<sup>th</sup> rev. ed. Natl. Acad. Sci., Washington, DC.
- Neuwirth, J. G., J. K. Norton, C. A. Rawlings, F. N. Thompson, and G. O. Ware. 1979. Physiologic responses of dairy calves to environmental heat stress. *Int. J. Biometeorology* 23:243-254.

- Nonnecke, B.J., M.R. Foote, B.L. Miller, M. Fowler, T.E. Johnson, and R.L. Horst. 2009. Effects of chronic environmental cold on growth, health, and select metabolic and immunologic responses of preruminant calves. *J. of Dairy Sci.* 92:6134–6143.
- Olson, D.P., C.J. Papasian, and R.C. Ritter. 1980. The effects of cold stress on neonatal calves. I. Clinical condition and pathological lesions. *Can J Comp Med.* 44:11–18.
- Perez, E., J.P.T.M. Noordhuizen, L.A. van Wuijkhuise, and E.N. Stassen. 1990. Management factors related to calf morbidity and mortality rates. *Livest. Prod. Sci.* 25:79–93.
- Reed, R. H., J. W. B. Allen I. Laskin, and M. G. Geoffrey. 2004. The inactivation of microbes by sunlight: Solar disinfection as a water treatment process. *Adv. in App. Microbiology.* 54:333-365.
- Reynolds, L. P., C. L. Ferrell, J. A. Nienaber, and S. P. Ford. 1985. Effects of chronic environmental heat stress on blood flow and nutrient uptake of the gravid bovine uterus and foetus. *J. Agric.Sci.* 104:289–297.
- Schmidt-Nielson, K. 1997. Temperature, Heat and Heat Transfer Pages 247-252 In *Animal Physiology: Adaptation and environment.* Cambridge University Press, New York.
- Scibilia, L.S., L.D. Muller, R.S. Kensinger, T.F. Sweeney, and P.R. Shellenberger. 1987. effect of environmental temperature and dietary fat on growth and physiological responses of newborn calves. *J. of Dairy Sci.* 70:1426–1433.
- Shearer J.K., D.K. Beede, R.A. Bucklin and D.R. Bray. 1991. Heat stress. Part 3. Nutritional management of dairy cattle during hot weather. *Agri-Practice.* 12 (5) reprint.
- Silanikove, N. 2000. Effects of heat stress on the welfare of extensively managed domestic ruminants. *Livest. Prod. Sci.* 67:1–18.
- Smith, B.P. 2009. *Large animal internal medicine.* 4th ed. St. Louis: Mosby, 2009.
- Spain, J. N. and Spiers, D. E. 1996. Effects of supplemental shade on thermoregulatory response of calves to heat challenge in a hutch environment. *J. Dairy Sci.* 79:639–646.

- St-Pierre, N. R., B. Cobanov, and G. Schnitkey. 2003. Economic losses from heat stress by us livestock industries. *J. Dairy Sci.* 86:E52-77.
- Stanton, A. 2009. Challenges and opportunities for managing respiratory disease in dairy calves. *Anim. Health Res. Rev.* 10:113–115.
- Stermer, R. A., C. F. Brasington, C. E. Coppock, J. K. Lanham, and K. Z. Milam. 1986. Effect of drinking water temperature on heat stress of dairy cows. *J. Dairy Sci.* 69: 546-551.
- Stilwell, G. and R. C. Carvalho. 2011. Clinical outcome of calves with failure of passive transfer as diagnosed by a commercially available IgG quick test kit. *The Canadian Vet. J.* 52:524-526.
- Tao, S., J. W. Bubolz, B. C. do Amaral, I. M. Thompson, M. J. Hayen, S. E. Johnson, and G. E. Dahl. 2011. Effect of heat stress during the dry period on mammary gland development. *J. Dairy Sci.* 94:5976–5986.
- Tao, S., A. P. A Monteiro, I. M. Thompson, M. J. Hayen, and G. E. Dahl. 2012. Effect of late- gestation maternal heat stress on growth and immune function of dairy calves. *J. Dairy Sci.* 95:7128-7136.
- Tizard, I. R. 2009. *Veterinary Immunology: An Introduction*. 8<sup>th</sup> rev. ed. Saunders Elsevier, St. Louis, MO.
- Walz, P.H., T.P. Mullaney, J.A. Render, R.D. Walker, T. Mosser, and J.C. Baker. 1997. Otitis media in preweaned Holstein dairy calves in Michigan due to *Mycoplasma Bovis*. *J. Vet. Diagn. Invest.* 9:250–254.
- Winchester, C. F., and M. J. Morris. 1956. Water intake rates of cattle. *J. Anim Sci.* 15: 722-740.
- Wu, G., F. W. Bazer, J. M. Wallace, and T. E. Spencer. 2006. Board invited review: Intrauterine growth retardation: Implications for the animal sciences. *J. Anim. Sci.* 84:2316–2337.
- USDA. 2007. *Dairy 2007, Part I: Reference of Dairy Cattle Health and Management Practices in the United States*. USDA-APHIS-VS, CEAH. Fort Collins, CO.
- West, J. W. 2003. Effects of heat-stress on production in dairy cattle. *J. Dairy Sci.* 86: 2131-2144.